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RELATION OF SOIL TEMPERATURE AND OTHER FACTORS TO ONION SMUT INFECTION

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OCCURRENCE OF ONION SMUT IN RELATION TO CLIMATE AND CULTURAL PRACTICES

The onion smut fungus, *Urocystis cepulae* Frost, was first reported by Ware (*11*) [1] in the Connecticut River Valley in 1869. At this early date it was causing some injury to the onion crop, and in 1888 it was reported by Thaxter (*10*) to be of much importance in the old onion soils of southern New England. During the years which have since elapsed it has successively appeared and become an economic factor in nearly all the more westerly regions of intensive onion culture of the northern States, from New York to Oregon. It is possible that this fairly rapid distribution of the parasite has been occasioned to some extent by smut spores carried with the seed, as already noted by Chapman (*2*) and Munn (*7, p. 412*). It has, however, more probably been brought about by the increasingly widespread distribution of onion sets. Many of these sets are grown in the northern States on smut-infested soils, and since they are shipped in quantity to all parts of this country, and even exported, their rôle in the wide dissemination of smut spores is obvious.

Chance introduction of the smut fungus in this way in the northern commercial onion-growing sections seems almost certain to lead to its permanent establishment. This evidently results from the fact that the common intensive practice of continuous cropping with onions for an indefinite term of years tends, when once the inoculum is introduced in the soil, to favor its increase and wider distribution season by season until it becomes a factor limiting further success with this crop. While this holds true for the northern States, it does not seem to be so in the southern sections. This is the more noteworthy since northern sets grown on smutty soil are each year shipped into the southern onion districts for propagation. This regional limitation of the smut fungus was impressed upon one of the authors (Walker) in connection with a

[1] Reference is made by number (italic) to "Literature cited," p. 261.

survey which he made some two years ago of the chief onion-growing centers of the entire country, as a representative of the Office of Cotton, Truck, and Forage Crop Disease Investigations of the United States Department of Agriculture. In connection with this, he personally inspected the leading onion-growing sections of Texas and Louisiana and conferred with the plant pathologists of these two States, Drs. C. W. Edgerton and J. J. Taubenhaus. No evidence of the disease was found, and it had not been reported to the Experiment Station of either State.[1]

In comparing the distribution and occurrence of onion smut in different sections of the country, it is necessary to keep in mind that two distinct types of onion culture are practiced in the United States. The first is followed in practically all of the northern sections, the second is the rule in the southern commercial growing regions, and in one or more sections in the Pacific coast States. In the first, or northern, type the seed is sown directly in the field as early in the spring as the soil can be properly prepared—that is, in March, April, or May, according to local climatic conditions. The bulk of the crop is harvested in these northern districts in August and September. The Globe varieties predominate, including the red, yellow, and some white. In the second, or southern, type of culture the seed is sown in special beds in late summer. The seedlings are then transplanted to the main field during the early winter months and the crop is harvested during the period from April to July. Here the Bermuda, Italian, and Spanish varieties predominate. The survey previously referred to brought out the fact that onion smut has become established in essentially all of the older onion-growing sections, which practice the first type—with spring sowing of seed—while smut is either entirely unknown or of no economic importance in those localities where the seed is sown in summer followed by transplantation. Wherein lies the explanation? As already suggested, it can not be due to the matter of chance introduction of the organism. This is certainly being distributed frequently and widely throughout the South. It would seem rather to be associated with some of the factors incident to the southern type of culture. The conspicuously different factors as outlined above are three: (1) The varieties used, (2) transplantation vs. direct seeding, (3) climatic differences associated with season of culture.

Greenhouse experiments, in which we have tested the different types, have shown that the Bermuda and Spanish varieties which are used in the South are as susceptible to smut infection as are the Globe varieties of the northern sections. Hence varietal resistance does not furnish the explanation. Turning to cultural methods, we find that in the South the seed beds in which the onions are grown preliminary to transplantation are usually of considerable size and are located as a rule in a portion of

[1] The authors are indebted to Doctors Edgerton and Taubenhaus for continued cooperation in the search for the smut in their respective States. They each reported again early in the current year that not a single specimen had as yet been found.

the main field. Therefore, if the organism were present and environing factors were favorable, it does not seem probable that this method of culture would completely inhibit the disease. Indeed, judging from our experience with cabbage transplantation in relation to clubroot and other soil- or seed-borne diseases, this method, instead of reducing the trouble, is likely to serve as a ready means of distributing the parasites with diseased seedlings from localized centers to wider areas. We are thus forced to turn for explanation of the absence of smut in the South to the third suggestion, that relating to climatic differences, bearing in mind the respective cultural seasons. The most evident environmental differences associated with the two types of culture relate to soil temperature and moisture during the time of seed germination and early seedling development, which constitute the smut infection period. In the northern type, the spring-sown seed develops in a soil which is comparatively cool and which has in general a relatively high and constant surface moisture content. In the southern type, the summer-sown seed must germinate and pass the early developmental stages in a soil of relatively high temperature and subject to superficial desiccation. Our problem has, therefore, necessitated an attempt to analyze and evaluate the possible factors associated with variations in soil moisture and soil temperature during the seedling stage.

INFECTION PERIOD

It has been of obvious importance in this study to know quite definitely the period in the development of the host at which infection actually occurs. Thaxter (*10*) gave critical attention to the time and manner of infection, concluding that the fungus always invaded the young seedlings below the surface of the soil and that, by subsequent growth of the host, the infected cells were commonly carried above the ground before visible signs of the disease appeared. He also noted that onion sets and onion bulbs replanted for seed growing were not attacked and suggested that the seedling was probably subject to attack in only the early stages of its development. Sturgis (*9*) later found that seedlings half as thick as a lead pencil and about 5 inches high, transplanted into smutty soil did not contract the disease. Sirrine and Stewart (*8*), in an experiment started at Jamaica, N. Y., on May 2, sowed eight rows of onion seed, each 10 feet in length, in soil free from smut. Alternate rows were left as controls. Soil from a smut-infected field was introduced in three ways: (1) in the furrow with the seed in two rows; (2) on the surface of the soil after the furrow was closed in one row; and (3) in a fourth row, on the surface of the soil 11 days after planting, this being shortly after the seedlings appeared above ground. The control rows remained healthy. Heavy infection occurred with the first treatment, slight infection with the second, no infection with the third. It seems possible

that in the third treatment, where the inoculum was merely placed on the surface of the soil when the seedlings were well started, the method failed to insure a sufficiently intimate and immediate contact of germinating spores with embryonic tissue to justify definite conclusions. Reviewing the evidence as a whole, however, it is obvious that the smut fungus is capable of invading the onion seedling for only a short period after seed germination.

In order to define more exactly the limits of this period of smut infection, we carried through a series of greenhouse trials. In the first of these 17 pots of sterilized greenhouse soil were planted with Red Globe onion seed which had been treated with 1 to 25 formaldehyde solution for 15 minutes. At two-day intervals beginning the eleventh day after planting, two pots were inoculated by mixing smut-infested soil in the upper layers of the pot, so that the inoculum was brought into close contact with the embryonic region of the cotyledon. At the time of inoculation, all retarded seedlings were removed, so that only plants of uniform height were considered in each case. All plants were pulled and examined for signs of the disease three to four weeks after inoculation. The results of this experiment, given in Table I, show that, under greenhouse conditions at least, infection may occur until the cotyledon is about 2½ inches above ground, or for a period of two weeks or more after sowing. Thus, the infection period appears to be slightly longer than that reported by Sirrine and Stewart (*8*).

TABLE I.—*Relation of the stage of development of the onion seedling to infection by Urocystis cepulae*

Soil treatment.	Pot No.	Length of period between sowing and inoculation.	Height of cotyledons above ground at inoculation.	Number of plants.	Percentage infected.
		Days.	*Inches.*		
Inoculated	1	11	1–1½	28	89
	2	11	1–1½	18	67
	3	13	1½–2	24	21
	4	13	1½–2	19	26
	5	15	1½–2	17	53
	6	15	1½–2	16	75
	7	17	2 –2½	19	16
	8	17	2 –2½	20	25
	9	19	2½–3	11	00
	10	19	2½–3	11	00
Uninoculated	11	.....	.....	14	00
	12	.....	.....	37	00
	13	.....	.....	22	00
	14	.....	.....	21	00
	15	.....	.....	18	00
	16	.....	.....	25	00
	17	.....	.....	45	00

The foregoing experiment was repeated in a somewhat cooler house, in which the temperature remained close to 15° C. most of the time, rising to about 20° during the middle of the day. Under these conditions, the maximum length of the cotyledon was about 2½ inches. The data from this experiment are reported in Table II. The plants became immune at approximately the same time as noted in the first experiment—between the nineteenth and twenty-fourth days after sowing, when the cotyledon had about attained its full growth and as the first leaf was emerging. It will be recalled that the basal portion of the cotyledon, as with each of the later leaves, forms a collar or sheath inclosing the lower parts of the younger leaves. The question arose as to whether or not immunity to smut infection is directly associated with maturity of the tissues. If so, it would seem that the explanation of this later escape of the onion plant from infection lies in the fact that the maturing basal sheath forms a thin but normally complete barrier of resistant tissue between the potentially infective soil and the deeper-lying embryonic tissues of the younger developing leaves. The removal of this mechanical barrier might, therefore, permit of infection at a later stage. In order to determine whether this is the case, the following experiments were undertaken. After the thirty-first day, when the onion seedlings had passed the so-called susceptible period, the cotyledons were carefully removed from the plants in one pot, and infected soil was placed around the base of the exposed first leaf. Sixty per cent of the plants thus treated became infected as shown in Table II, pot 9. This proves that the first leaves are susceptible even after the cotyledon becomes immune. On the fortieth day, a 1-inch layer of infested soil was placed on top of pots 10 and 11, so as to surround the first leaves in proximity to the axils. Pot 10 was left at the same temperature (15° to 20°) for 24 days and pot 11 was removed to a temperature of 25° to 28° for the same period. About 5 per cent of the plants in pot 10 showed infection of the first leaf as compared with 28 per cent in pot 11. The reason for the increased percentage of infection at the higher temperature has not been satisfactorily explained. It may simply have been consequent upon the stimulated growth of the onion foliage. However this may be, it is evident that the basal portion of the first leaf remains susceptible to infection for a short time, at least, after it emerges from the cotyledon.

From a summary of these results it appears that our own experimental data regarding the duration of the period of infection agree in the main with those of previous investigators. The conclusion seems justified that disease resistance is correlated with tissue maturity, and that the onion cotyledon becomes immune to smut infection at about the stage when growth ceases. The rate and nature of growth of the cotyledon will naturally vary with environmental conditions; hence variation in the actual length of the infection period is to be expected. The mature

basal sheath of the cotyledon thus protects the embryonic region of the younger leaves from infection. That portion of the first leaf which emerges from the cotyledon is susceptible to infection for some little time after emergence, but since it ordinarily is not actually in contact with infested soil, this fact is probably not of practical significance.

TABLE II.—*Relation of stage of development of the onion seedling to infection by Urocystis cepulae*

Pot No.	Method of exposure to inoculation.	Interval between sowing and inoculation.	Length of cotyledon at time of inoculation.	Condition of first leaf at time of inoculation.	Interval between inoculation and final examination.	Total number of plants.	Percentage smutted.
		Days.	*Inches.*		*Days.*		
1–3	Uninoculated.....	.....	.....	.....	.....	100+	0
4	Infected soil around base of cotyledon.	13	1	Not out.....	27	24	75
5	Do.....	16	1½	...do.....	24	34	35
6	Do.....	19	2 to 2¼	...do.....	24	41	8
7	Do.....	24	2 to 2¼	Just out....	24	19	0
8	Do.....	31	2 to 2¼	½ to 1½ inches above axis.	24	36	0
9	Cotyledons removed and infected soil placed around base of first leaf.	31	2 to 2¼	...do.....	24	15	60
10	One-inch layer of infected soil placed on surface of old soil so as to cover lower inch of aerial portions of plants.	40	2 to 2½	½ to 4 inches above axis.	24	36	6
11	Same as No. 10, except that pot was transferred to a temperature of 25° to 28° C.	40	2 to 2½	...do.....	24	46	28

RELATION OF SOIL MOISTURE TO INFECTION

Since there are these well-defined limits to the time of smut infection, the possibility becomes clearly evident that variable environmental factors during this limited period may exercise a controlling influence on the occurrence of the disease. As already stated, the problem seems to resolve itself primarily into the question of the relations of soil temperature and soil moisture to infection. The results of several workers upon the grain smuts, as summarized by Jones (5), have shown that soil temperature may influence infection. Hungerford and Wade (4) have published evidence that high soil moisture, also, may favor infection of wheat by the smut fungus *Tilletia tritici* (Beij.) Wint. Variations in the moisture content of the surface layers of soil are likely to be wide, especially during the late summer planting season in the southern States when high temperatures and low humidity may cause rapid desiccation.

An experiment was therefore carried out in which onion seedlings were grown in smut-infested soil in pots which were held at different degrees of soil moisture. Galvanized iron pots 5 inches in diameter and 4 inches deep were used for these trials. Greenhouse sandy loam soil was used and its water-holding capacity was determined in advance by the two standard methods recommended by soil physicists [1]—that is, by means of the 10-inch cylinder and the 1-cm. cup. The soils were brought to the desired low and medium water contents before they were placed in the culture pots; and in those cases where the desired moisture approached the water-retaining capacity, the water content was finally adjusted after the soil was potted. Although these methods failed to secure exact uniformity in the physical compactness of the soils in the several series, they were considered satisfactory as to initial moisture conditions. The pots were weighed daily during the progress of the experiment, and water was added to replace the losses. Since it was realized that the surface layer of soil would change in moisture content through evaporation more rapidly than the lower layers, an effort was made to reduce this surface evaporation so far as practicable. To this end, tar paper covers were used until the seedlings came above ground, when glass covers were substituted for a few days, and finally mineral wool was packed on the surface about the seedlings to reduce evaporation. Absolutely uniform moisture throughout the pot could not be maintained even by this method, and the upper layers of soil unavoidably assumed a somewhat lower water content than the average for the pot. Therefore, at the end of the experiment, moisture determinations were made of the upper inch of soil, since this was the important part from the standpoint of smut infection.

The soil was inoculated at the outset by the introduction of spores from diseased leaves and scales. The data from this experiment are given in Table III. Good germination took place within the range of 10 to 15 per cent moisture content (45 to 70 per cent of the moisture-holding capacity). A high percentage of infection also occurred within this range. Above 15 per cent there was some decrease in germination together with a gradual reduction in infection. At the extreme, however, where germination of seed was practically eliminated (pot 1), one of the two plants surviving became infected. It is evident from these data that a good percentage of infection may be expected at a soil moisture content up to the limit where good germination and growth of the host plant occur. There was a gradual reduction of infection below 5 per cent (23 per cent of the moisture-holding capacity), but this was not sufficient to insure good germination and support good growth. It may be concluded, therefore, that soil moisture does not function as a factor limiting infection with onion smut within the limits at either extreme where good germination and growth of the host occur.

[1] We are especially indebted to Prof. E. Truog, of the Department of Soils of the University of Wisconsin for advice in connection with this work.

TABLE III.—*Relation of soil moisture to infection by Urocystis cepulae*

Pot No.	Original moisture content (percentage of dry weight).[a]	Moisture content of surface layer at end of experiment.	Number of seeds planted.	Total number of plants.	Percentage of plants smutted.
1	19.6	27.1	100	2	50
2	17.1	24.7	100	8	0
3	16.3	23.7	100	52	42
4	18.2	23.4	100	48	71
5	20.0	14.0	100	50	90
6	15.0	12.4	100	60	97
7	15.0	11.6	100	66	98
8	10.0	9.3	100	66	98
9	10.0	9.0	100	71	85
10	10.7	2.4	100	66	94
11	5.0	2.5	100	32	59
12	8.1	1.4	100	66	50
13	7.5	3.4	100	63	19
14	6.6	1.3	100	37	35

[a] Water-holding capacity, as determined by 10-inch cylinders, was 22.3 per cent; as determined by the 1-cm. cup, it was 27.8 per cent.
The calculated wilting coefficient of the plants was 2.3 per cent.

TEMPERATURE RELATIONS

In calling attention to the importance of soil temperature as a factor in the development of certain plant diseases, Jones (5) points out that several investigators have stressed its bearing upon infection in the case of stinking smut of wheat, *Tilletia tritici* (Beij.) Wint., and of the oat smuts, *Ustilago avenae* (Pers.) Jens. and *Ustilago levis* (K. and S.) Mag. Heald and Woolman (3) showed that the amount of infection with the stinking smut of wheat was reduced as the mean soil temperature rose above 65° F. (18.3° C.) or fell below 40° F. (4.4° C.). Humphrey (4) states, for the same disease, that soil temperatues of 0° to 5° C. and above 22° C. are decidedly unfavorable to infection.

In studying the relations of soil temperature to the developemnt of a parasitic disease, consideration must be given to the possible influence of such temperature upon the host and the parasite independently. This may enable one to analyze with more confidence the effects when host and parasite are subjected simultaneously to the experimental condition. This has been done as far as practicable in connection with the present work.

Unfortunately, germination of the fungus spores under artificial conditions has been so scanty that the effect of temperature upon the fungus has been necessarily limited to inoculation experiments with infested soil. However, the disease is produced so readily and consistently by the latter method that a very accurate index to the development of the fungus can be secured by varying the condition of the infested soil during the germination and early growth of the onion seedling.

The soil-temperature experiments were all carried out in the greenhouse at Madison, Wis., during the winter months. The apparatus in use

in the Department of Plant Pathology, University of Wisconsin, for the control of soil temperatures has been described by Jones (5). Briefly, it consists of a series of water baths held at constant or nearly constant temperatures in which the glass or galvanized-iron culture pots are inserted.[1]

For these experiments galvanized-iron cylindrical pots 5 inches in diameter and 8 inches in depth were used. In order to overcome the influence of the air temperature upon the upper layer of soil, the surface of the latter was kept at ½ to 1 inch below the level of the water. Tar-paper covers were placed over the pots until the seedlings came above ground; these covers were then removed, and a layer of mineral wool was placed on the surface of the soil. By this procedure the temperature of the upper inch of soil was kept reasonably close to that of the deeper portions—that is, approximately that of the water in the tank. In order to follow any minor variations, readings were taken three times daily from thermometers inserted 1 inch below the surface of the soil. At the beginning of the experiments the moisture content of the soil was adjusted to two-thirds of the water-holding capacity. The pots were thereafter weighed at intervals of one to three days, depending upon the rate of water loss, and the moisture content was readjusted accordingly, either by adding water directly to the surface or by introducing it at the bottom of the pot through a glass tube. Obviously this method did not secure uniform distribution of moisture throughout the pot, and unavoidably the content of the upper layer of soil was somewhat lower than the average for the whole pot. It is believed that this variation had little if any influence, however, since other experiments, described earlier in this paper, showed that infection is quite uniform over a much wider range of soil moisture than here occurred. The seed was planted at a depth of 1 inch. Since the chlorophyll in the tops in some cases obscured the smut lesions, the plants were placed in alcohol acidified with acetic acid until thoroughly bleached before final examination for the disease was made.

EFFECT OF SOIL TEMPERATURE UPON THE DEVELOPMENT OF THE HOST

Experimental studies to determine the relation of soil temperature to the rate and character of seed germination and seedling development were carried on in conjunction with those relating to infection, of which the results will be presented in the next section. It will be simpler, however, to discuss these two aspects of the problem separately, taking up first the relations of temperature to host development.

EXPERIMENT I.—Seven pots of sterilized greenhouse loam soil were uniformly planted with 50 Red Globe onion seeds in each pot. One pot was then held at each of the following temperatures: 10° to 14°, 16.5°

[1] Since this description was published, numerous improvements have been made from time to time by members of the Department. As now in use these are termed the "Wisconsin soil temperature tanks."

to 18°, 19° to 22°, 24° to 26°, 27° to 29°, 30° to 31°, 35° C. The moisture content of the soil was held at two-thirds the water-holding capacity (22 per cent of dry weight). The air temperature of the greenhouse was kept at about 15° with a rise to 20° during the middle of the day. These conditions as to soil moisture and air temperature were such as had proved favorable for both host and parasite development in the earlier trials. The first seedlings to appear above ground were those at 27° to 29°, those at 24° to 26° came up shortly afterward, then those at 19° to 22°. Good growth took place at these three temperatures, but germination was very slow at lower temperatures. At the highest temperature, 35°, a few seeds germinated, but growth was very slight. The plants were all removed and the roots washed out on January 7, 1920, 29 days after the seed was sown. The data given in Table IV summarize the condition of the plants at this date. It will be seen that at this early stage in the development of the plants there was a tendency for best root development at about 21° or below, while the best development of tops took place at this point or above.

TABLE IV.—*Development of onion seedlings in sterilized greenhouse soil held at 22 per cent of the dry weight or two-thirds the moisture-holding capacity, and at different soil temperatures. Data on January 7, 1920, 29 days after sowing*

Soil temperature.	Number of seeds planted.	Total number of plants.	Percentage with 2 roots.	Percentage with 3 roots.	Percentage with first leaf.	Average dry weight of tops per plant.	Average dry weight of roots per plant.	Average total dry weight per plant.
°C.						Gm.	Gm.	Gm.
10 to 14	50	41	22	0	17	0.00224	0.00046	0.0027
16.5 to 18	50	30	17	3	70	.00293	.00023	.0031
19 to 22	50	32	56	25	100	.00396	.00043	.0044
24 to 26	50	28	32	0	82	.00307	.00028	.0033
27 to 29	50	[a] 14	50	0	86	.00285	.00014	.0030
30 to 31	50	[a] 8	13	0	63	.00212	.....	.0021

[a] The reduced stand at 27° to 29° and 30° to 31° C. was due to damping-off fungi.

EXPERIMENT II.—The experiment was repeated, with some modifications, starting April 10, 1920. The Red Globe and Yellow Bermuda varieties were used. Two pots of each variety were kept at each of the following soil temperatures: 14°, 20°, 25°, 28°, 30° C. The air temperature ran slightly higher (20° to 30°) during the middle of the day and dropped to about 15° for the most of the night. Both the rate and the percentage of germination were noted, and the data are recorded in Table V. In both varieties the most rapid germination took place at 25°, although the rate was only slightly less at 20°, 28°, and 30°. At 14° the seedlings were distinctly slower in starting off. The plants from one pot of each series were removed on the thirteenth day. The dry weights of the tops and roots as given in Table V were so small at this age that comparison on this basis does not have any great value. The tendency for rapid development of tops as compared with roots at 20° or above is, however, shown very strikingly in Plate 25. The plants in the remaining

pots were removed on the thirtieth day. The relative dry weights of roots and tops then secured are shown in Table V and those of the Red Globe are graphed in figure 1.

While the temperature relations of the two varieties were alike in their main features, there was an interesting minor difference, possibly indicative of the better adaptation of the Red Globe for northern culture and of the Yellow Bermuda for southern. In both cases with these onions, as indeed holds generally in our experiments with other plants, the best

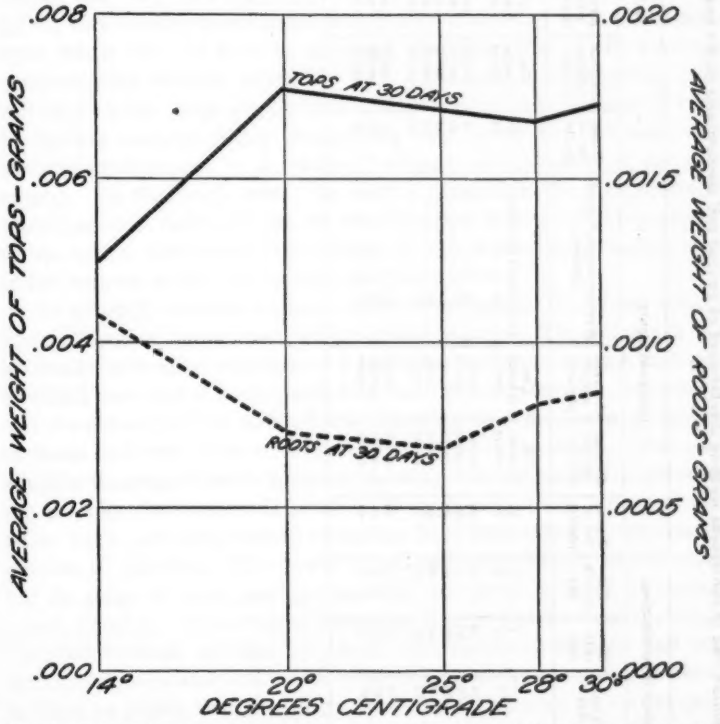

FIG. 1.—Relative developments of dry weight in tops and in roots of Red Globe onion as shown at end of 30 days' growth in a series of culture pots kept at the several soil temperatures indicated, with all other factors, including air temperature, alike for all. Note that the best root development occurs at the low temperatures (12° to 15° C.) whereas the tops are forced more strongly at higher temperatures (20° to 25°).

root development occurs at relatively lower temperatures (12° to 20° C.) while best top development occurs at higher temperatures (20° to 25°). When the varieties are compared, it is seen that with the Globe both roots and tops grew relatively better at somewhat lower temperatures than did those of the Bermuda. Thus the data at 30 days show the maximum root development of the Globe at 14° while that of the Bermuda was at 20°; with tops the maximum was at 20° for the Globe and at 25° for the Bermuda.

TABLE V.—*Rate of onion seed germination and of subsequent seedling development at various soil temperatures with a uniform air temperature of 15° to 20° C.*

Variety.	Soil temperature.	Percentage of germination.				13-day-old plants.				30-day-old plants.			
		Seventh day.	Ninth day.	Eleventh day.	Thirteenth day.	Number of plants.	Average weight of tops.	Average weight of roots.	Average stage of development.	Number of plants.	Average weight of tops.	Average weight of roots.	Average stage of development.
	°C.						Gm.	Gm.			Gm.	Gm.	
Red Globe	14	0	0	7	59	38	0.00126	0.00026	Cotyledon; first root	60	0.00499	0.00108	First leaf; third root.
	20	25	65	73	73	70	.00175	.00037	do	67	.00709	.00073	Second leaf; first and second roots.
	25	63	72	74	74	56	.00180	.00033	do	44	.00684	.00068	Second leaf; first root.
	28	52	66	68	70	60	.00186	.00029	do	49	.00672	.00081	Second leaf; first root.
	30	48	65	67	69	57	.00168	.00030	do	45	.00691	.00085	Second leaf; first root.
Yellow Bermuda	14	0	0	22	56	54	.00133	.00040	do	46	.00717	.00108	First leaf; third root.
	20	37	63	66	68	45	.00180	.00040	do	68	.00766	.00122	Second and third leaf; second and third roots.
	25	54	64	65	65	49	.00190	.00044	do	41	.00896	.00119	Second leaf; first root.
	28	43	53	56	56	32	.00175	.00037	do	33	.00569	.00084	Second leaf; first root.
	30	49	58	59	61	40	.00182	.00040	do	45	.00597	.00075	First and second leaves; first root.

EFFECT OF SOIL TEMPERATURE UPON INFECTION

At present the chief interest in these data focuses upon the question of any possible bearing of the rate of development of the host plant at different temperatures upon predisposition to, or escape from, smut infection, recalling that such infection is practically limited to the seedling stage before the maturity of the cotyledon. It has just been noted that the promptest seed germination and most rapid growth of tops during this early seedling stage occur at fairly high temperature, 20° to 25° C., with a rather pronounced drop in rate of aerial growth at temperatures below 20°. It is to be expected, therefore, that in the northern onion-growing sections where the seed is planted in early spring there will be a rather tardy germination and slow early development of tops, the growth energies during the seedling stage being directed under this climatic environment to a relatively stronger development of the root system. In the South where the seed is planted in the comparatively warm period of early autumn, we should expect a more rapid top growth at the outset, with correlated strength of root development coming later in the autumn as the soil becomes gradually cooler.

The naturally infested soil was secured near Racine, Wis., from a badly diseased field of sandy loam rich in organic matter. The soil which was artifically inoculated consisted of a greenhouse mixture of loam and sand to which were added fresh spores from smutty onion leaves. In order to test the efficacy of this method of soil inoculation a preliminary planting of onion seed was made in advance of the final experiments. This gave a high percentage of smut infection, showing that the method of introducing the inoculum was satisfactory. Several early trials indicated that below 25° C. soil temperature variations have little effect on the relative amount of infection. The results of two such experiments, nearly covering the range of onion seed germination, are given in Table VI (experiments 1 and 2). It is evident from these figures that abundant infection occurred between 10° and 25°, both with naturally infested and with artificially inoculated soil. The number of pustules per plant as shown in Plate 25 proves that the fungus was very active even at low temperatures. Above 25° infection is reduced very rapidly, as indicated by both the percentage of infected plants and the number of pustules per plant.

In order to determine more closely the point at which infection is inhibited four more serial experiments were conducted (experiments 3 to 6) in which the temperature was kept as constant as possible at 2-degree intervals between 25° and 31° C. The results given in Tables VII and VIII, and illustrated in Plates 26 and 27, show that abundant infection took place at 25° to 26°, while it was greatly reduced at 27° to 28° and completely inhibited at 29° or above. The infected seedlings from experiment 5 show the great reduction in the amount of disease per

plant at 27° to 28° as compared with 25° to 26°. It is interesting to note that infection was reduced more abruptly at 27° to 28° in the artificially inoculated soil than in that naturally infested. This may be due to the age of the inoculum, a smaller percentage of the spores being functional in the former soil, or perhaps to the presence of a smaller amount of inoculum.

TABLE VI.—*Relation of soil temperature to infection of onion seedlings by Urocystis cepulae*

Experiment 1.			Experiment 2.		
Naturally infested soil. Begun May 3, 1919; completed May 30, 1919. Records not kept as to soil moisture nor as to air conditions.			Artificially inoculated soil. Begun Dec. 10, 1919; completed Jan. 6, 1920. Soil moisture held at 22 per cent or two-thirds the water-holding capacity. Air temperature 13° to 25° C., relative humidity 45 to 75 per cent.		
Soil temperature.	Total number of plants.	Smutted plants.	Soil temperature.	Total number of plants.	Smutted plants.
°C.		*Per cent.*	°C.		*Per cent.*
10 to 13	25	72	10 to 14	64	98
18 to 20	5[a]	80	16.5 to 18	49	98
22 to 24	6[a]	100	19 to 22	63	100
25 to 30	47	15	23 to 26	56	93
28 to 34	25	0	27 to 29	52	8
			29 to 31	36	0

[a] Stand reduced by damping-off fungi.

TABLE VII.—*Relation of soil temperature to infection of onion seedlings by Urocystis cepulae*

Experiment 3.			Experiment 4.		
Naturally infested soil. Begun Dec. 20, 1919; completed Jan. 10, 1920. Soil moisture held at 25 per cent or two-thirds the water-holding capacity. Air temperature, 13° to 25° C.; relative humidity, 45 to 75 per cent.			Artificially inoculated soil. Begun Dec. 18, 1919; completed Jan. 12, 1920. Soil moisture held at 13 per cent or two-thirds the water-holding capacity. Air temperature, 13° to 25° C.; relative humidity, 45 to 75 per cent.		
Soil temperature.	Total number of plants.	Smutted plants.	Soil temperature.	Total number of plants.	Smutted plants.
°C.		*Per cent.*	°C.		*Per cent.*
19 to 22	44	93	.....	.....	.....
23 to 26	131	96	23 to 26	50	98
26 to 27	100	57	26 to 28	58	12
27 to 28	86	12	29 to 31	40	0

TABLE VIII.—*Relation of soil temperature to infection of onion seedlings by Urocystis cepulae*

Experiment 5. Naturally infested soil. Begun Jan. 16, 1920; completed Feb. 10, 1920. Soil moisture held at 25 per cent or two-thirds the water-holding capacity. Air temperature, 13° to 25° C.; relative humidity, 40 to 80 per cent..			Experiment 6. Artificially inoculated soil. Begun Jan. 16, 1920; completed Feb. 10, 1920. Soil moisture held at 15 per cent or two-thirds the water-holding capacity. Air temperature, 13° to 25° C.; relative humidity, 40 to 80 per cent.		
Soil temperature.	Total number of plants.	Smutted plants.	Soil temperature.	Total number of plants.	Smutted plants.
°C.		Per cent.	°C.		Per cent.
25 to 26	89	100	25 to 26	106	90
27 to 28	103	47	27 to 28	98	8
29 to 30.5	77	0	29 to 30.5	47	0
30 to 32	43	0	30 to 32	30	0

It may be concluded from the foregoing experiments that a high percentage of infection may be expected up to 25° C., above which there is a rather abrupt reduction, leading to complete inhibition at 29°. There appears to be no lower limit of temperature for infection within the range where onion seeds will germinate and normal growth occur.

After it was clearly shown that no infection would take place at 29° C., the question arose as to how long seedlings must grow at this temperature to become entirely immune. It has been shown that at moderate temperatures the plant becomes immune in about 20 days, or at about the time when the cotyledon has reached its maximum growth. To determine whether or not this condition is altered when the plants are grown at 29°., several pots each of the naturally and the artificially inoculated soil were started off at this high soil temperature. Pots were then transferred from time to time to a lower temperature favorable for infection (15° to 20°) where they were held for about three weeks before they were examined for signs of the disease. The results of these experiments are summarized in Table IX.

It is quite evident that the amount of infection was markedly reduced by an exposure of 15 to 18 days at 29° C. Complete inhibition of infection by even more protracted exposure to this high temperature was not attained. However, where infection did occur there was usually not more than one lesion per plant, which in the majority of cases was so situated that subsequent infection of newly forming leaves would be impossible. It is thus quite certain that where seedlings develop at about 29° for the first 20 days the amount of damage from smut will be negligible, especially in an area where the amount of inoculum is slight.

TABLE IX.—*Effect of different soil temperatures upon onion smut infection. In all cases except the fifth, tenth, and eleventh, the pots were held for the stated period at 29° C., where infection was inhibited, then transferred to 15° to 20°, a temperature favorable for infection. In the fifth and tenth, where the continuous temperature was high, note that practically no smut developed; in the eleventh, where the soil temperature was continuously low, note that practically all the plants were smutted; in the remaining series, where the plants were transferred from the higher temperature (29°) to the lower (15° to 20°) after varying periods, note that long exposures at the higher temperature tended to reduce the amount of infection.*

Pot No.	Type of soil inoculation.	Length of exposure to 29° C.	Size of plants at time of removal to low temperature.	Extent of infection at end of experiment.		
				Total number of plants.	Percentage diseased.	Extent of infection.
		Days.				
1	Artificially inoculated soil.	11	Cotyledons 1 inch long.	16	94	63 per cent diseased first leaf.
2		15	Cotyledons 2 to 2½ inches long.	17	41	12 per cent diseased first leaf.
3		18	.....do.....	12	25	25 per cent diseased first leaf.
4		28	First leaves 1+ inches high.	9	11	11 per cent diseased first leaf.
5		35	Continuous exposure at 29°.	6	0	
6	Naturally infested soil.	12	Cotyledons 1½ to 2 inches long.	29	48	14 per cent diseased first leaf.
7		16	Cotyledons 2½ to 3½ inches long.	40	13	3 per cent diseased first leaf.
8		23	First leaves out in about one-half plants.	24	8	8 per cent diseased first leaf.
9		27	First leaves out in most plants.	16	13	6 per cent diseased first leaf.
10		38	Continuous at 29°.	34	3	0 per cent diseased first leaf.
11		0	Continuous at 15° to 20°.	76	99	Most of these plants died in cotyledon stage.

It is interesting to note also in this connection that continued exposure of onion roots to a temperature of 29° C. led to the gradual slowing up of growth. With the transfer of the pots to the lower temperature (15° to 20°) both root and top development were greatly stimulated. In attempting to correlate these results one must keep in mind the fact that in nature the temperature conditions under which the onions develop are much different from those in the experimental pots. Whereas in the pots the soil temperature is uniform throughout their depth, in the natural soil there is a gradual decrease in temperature at progressively greater depths. The temperature of the upper inch runs extremely high during the summer months because of direct exposure to the sun's rays, and this is the area critical for infection by onion smut. The young roots, on the other hand, as they develop progressively reach strata of lower temperature, which are more favorable for their growth.

EFFECT OF HIGH AIR TEMPERATURE UPON THE DEVELOPMENT OF SMUT

The experiments reported above in which the soil temperature was varied were carried on at an air temperature of 15° to 20° C. The latter is considerably lower than the air temperature which prevails in southern onion sections at the time when young seedlings are starting off. This is shown for one section (Laredo, Tex.) in Table XIII, where the mean air temperature is about 30° during most of the onion-planting time. The question arose as to what effect these high air temperatures might have upon the development of smut in the aerial portions of the plant. Five clay pots of onions in naturally infested soil were started off in a greenhouse running at about 25°. Previous observations had shown that the pustules become evident in the cotyledons on about the twefth day under these conditions. Accordingly the plants were allowed to grow at this temperature for nine days, at which time a few lesions were barely visible. In order to prove that good infection had already taken place, 10 plants were removed from infested soil and washed thoroughly in running water to remove any external inoculum, after which they were transplanted to clean soil. Within two days lesions were distinctly visible in these plants, and smut developed in 8 out of the 10.

As a control on this method of removing the external inoculum 11 plants grown in clean soil were moistened and covered thoroughly with infested soil. They were then washed in running water and transplanted to clean soil. No smut developed. On the ninth day after sowing, 4 of the 5 pots were removed to a greenhouse running at 30° to 33° C., one being allowed to remain at 25°. One pot was then transferred from the higher temperature back to 25° at the end of each the second, fourth, ninth, and fourteenth days. This exposure to the higher temperature resulted in a stimulation of host plant growth for a few days. When the plants were allowed to remain at this high temperature, however, for three weeks distinct stunting became evident, while more prolonged exposure resulted in death. The plants so transferred were allowed to continue growth at 25° for three weeks or more, when they were examined for the presence of smut. The final results are given in Table X. It was evident that the gradual elimination of smut which took place was proportional to the length of exposure to the higher temperature (29°). After 14 days of exposure only small lesions developed on 16 per cent of the onion plants, although presumably 80 per cent or more of these plants were originally infected while they were growing at the lower temperature. This experiment was repeated several times with practically the same results, namely, that exposure of plants bearing incipient infections to a temperature of 30° to 33° for 12 to 15 days almost entirely checked further development of the parasite.

TABLE X.—*Effect of high temperature, following infection, in inhibiting the further development of smut. The plants were from a series of pot cultures started at 25° C. and held there until incipient infection had occurred, then transferred for the period indicated to a high temperature, 30° to 33°, and finally brought back to the original 25°.*

Length of exposure to temperature of 30° to 33°.	Number of plants.	Percentage smutted.
None (25° throughout)	34	94
2 days	29	45
4 days	30	37
9 days	40	33
14 days	36	17

It is to be noted that in the experiments just reviewed potted plants were used. The entire pot, thus including roots and tops of the experimental plants, was exposed to the stated temperature condition.

The question then arose whether the results secured were due entirely to the effect of high air temperature upon the fungus or to an indirect effect of the changed conditions upon the metabolism of the host. In order to throw some light upon this point two experiments were conducted in which seedlings were grown in infected soil at three constant soil temperatures and each of two air temperatures, 25° and 30° to 33° C. The results (Table XI) at the lower air temperature (25°) coincided closely with those previously secured at air temperatures of 13° to 25°, inasmuch as abundant infection occurred when the soil temperature was 25°, while complete inhibition was attained at 30°. It is, however, significant to note, in comparison with the results in Table X, that, with the soil temperature held at 20° or 24°, the 30° to 33° air temperature did not greatly check the development of the disease. It appears, then, that roots as well as tops must be exposed to the inhibitive higher temperature, 30° to 33°, in order fully to check the parasite after incipient infection has taken place. This suggests that the inhibitory effect may be due in part at least to the influence of the environmental conditions upon the metabolism of the host and not entirely to a direct effect upon the fungus itself.

TABLE XI.—*Effect of different combinations of soil and air temperature upon onion smut infection*

Air temperature.	Soil temperature.	Experiment 1.		Experiment 2.	
		Number of plants.	Percentage smutted.	Number of plants.	Percentage smutted.
°C.	°C.				
25	20	23	100	85	95
	25	68	97	49	92
	30	42	0	22	0
30 to 33	20	[a]9	89	41	46
	24	73	86	50	60
	30	24	0	30	0

[a] Stand reduced by damping-off fungi.

EFFECT OF MODERATELY HIGH TEMPERATURES UPON SYSTEMIC INVASION OF THE PLANT

Thaxter (*10, p. 134*) observed that in some instances the smut fungus may infect and develop in the cotyledon without invading the first leaf, with the result that the plant eventually outgrows the disease. Observations lead us to believe that this may vary with different temperatures. It has been pointed out that at a temperature of about 25° C. the most rapid top growth of the onion seedling occurs, while at temperatures below 20° the top growth is much retarded. Two pots of infested soil were sown with onion seed and placed in greenhouses, one at 24° to 28°, with a maximum of about 36°, for one or two hours on sunny days, the other at 15° to 20°. A high percentage of cotyledon infection occurred in both pots. After 31 days 24 out of 29 plants at the high temperature were infected, but the pustules were all confined to the cotyledons and no infection of first leaves had developed, although the plants were now in the second leaf stage. At the low temperature, on the other hand, of approximately the same number of plants, only 9 had survived on the thirty-seventh day, and 8 of these showed infection in the second leaves. It appears, then, that rapid growth of tops at about 25° may result in a large percentage of plants outgrowing the disease after the cotyledons become infected. The results of successive field plantings, discussed in the next paragraph, seem to confirm this judgment. The importance of the practical bearings of this matter are such as to justify further critical attention.

EFFECT OF SUCCESSIVE PLANTINGS THROUGHOUT THE GROWING SEASON UPON INFECTION

The laboratory experiments described early in this paper have shown that onion smut infection is greatly reduced where a constant soil temperature of 27.5° C. is maintained during the susceptible period of the plant's growth, while a temperature of 29° thus applied completely inhibits infection. Moreover, as explained in the last paragraph, when plants are growing in infested soil with temperature of air and soil held at about 25°, although a high percentage of cotyledon infection may occur, there is a greater tendency than at lower temperatures for the plants to outgrow the disease, owing to the rapid growth of tops. These results combined to justify the expectation that successive field plantings of onion seed throughout the growing season might show considerable variations in the percentage of smut infection. In the onion field the soil temperature usually varies widely during 24 hours, often reaching a maximum considerably above 29° during the day and descending to a minimum much below this at night. Under Wisconsin conditions the daily mean temperature gradually rises during the spring and early summer months and falls during the latter part of the growing

season. It seemed possible, therefore, that by making successive plantings a period might be found for this latitude when the mean soil temperature is sufficiently high to materially check or completely inhibit onion smut infection.

In order to test this out, a series of plantings at intervals of from 8 to 14 days was begun on June 18, 1920, at Madison, Wis. Onion seed was sown in smut-free soil in an open trench about 1 inch deep and was then covered with naturally infested soil similar to that used in certain of the laboratory experiments. Two varieties, Red Globe and Yellow Bermuda, were used, one 10-foot row of each variety being put in at

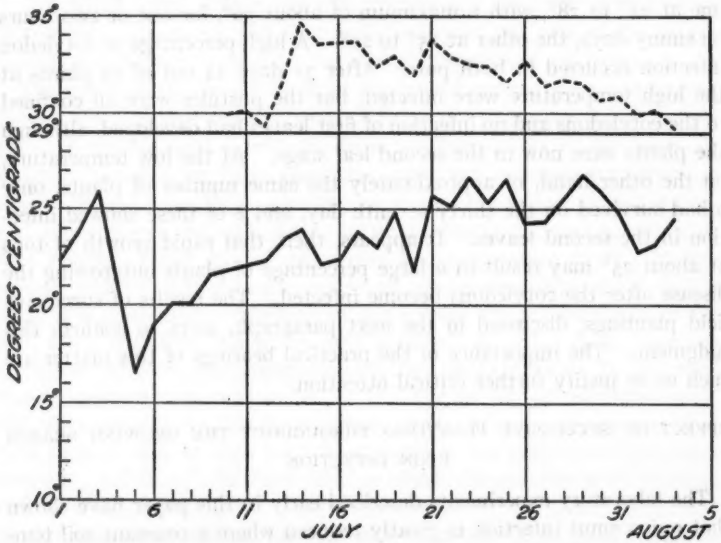

FIG. 2.—Graph showing the daily mean soil temperature at a depth of 1 to 2 inches as it occurred in the "successive planting" plots. Since the weather continued rather cool, one bed was covered with glass to insure a higher temperature. The temperature of the uncovered bed is shown by the solid line, the temperature of the glass-covered bed by the broken line. For further details see Table XII and and accompanying text.

each planting. Temperatures of the soil at a depth of 1 to 2 inches were obtained by means of a self-recording thermograph. The hourly mean temperature for each day was then secured by adding temperatures as recorded for each hour and dividing the sum by 24. These computations are represented graphically in figure 2. Since the weather in July was unusually cool, a higher mean soil temperature was secured for some of the plots by covering them with an ordinary glass cold frame. Inasmuch as the dry weather and high temperature would cause a rapid desiccation of the surface layer of soil, the plots were watered thoroughly on alternate days or oftener during the early growth of the plants. The

data collected from this field plot are tabulated in Table XII. Observations were made by pulling plants at several points in each plot, and examining for smut lesions after the chlorophyll had been removed by means of alcohol and acetic acid. The first observation was made on the twenty-first to the twenty-third day after planting. Subsequent observations were made as indicated in the table.

TABLE XII.—*Development of onion smut in successive plantings in the field at Madison, Wis., 1920*

Date of planting, 1920.	Variety.	Treatment.	First observation, 21 to 23 days after planting.		Second observation, 29 to 31 days after planting.		Third observation, 44 to 65 days after planting.		
			Number of plants examined.	Percentage smutted.	Number of plants examined.	Percentage smutted.	Time after planting (days).	Number of plants examined.	Percentage smutted.[g]
June 18	Red Globe	Uncovered	108	53	[b] 50	[c] 40	52	31	3
June 26	Red Globe	...do.....	62	73	51	[d] 76	44	51	39
	Yellow Bermuda.	...do.....	45	49	42	[e] 86	.....	.....	.....
July 10	Red Globe	...do.....	41	10	47	[f] 23	65	61	0
	Yellow Bermuda.	...do.....	20	10	28	[f] 7	65	24	0
July 19	Red Globe	Covered ..	52	0	33	0	65	9	0
	Red Globe	Uncovered	40	13	.....	.....	53	135	11
	Yellow Bermuda.	...do.....	35	14	.....	.....	53	39	13
	Red Globe	Covered [a].	46	0	.....	.....	53	91	9
	Yellow Bermuda.	Covered [a].	26	0	.....	.....	53	45	24

[a] Covered for 15 days only.
[b] Observation 39 days after planting.
[c] Extent of infection: Systemic, 26 per cent; confined to dead cotyledon, 14 per cent.
[d] Extent of infection: Systemic, 10 per cent; confined to dead cotyledon, 66 per cent.
[e] Extent of infection: Systemic, 5 per cent; confined to dead cotyledon, 81 per cent.
[f] Extent of infection: All cotyledon infections.
[g] Extent of infection: All systemic infections.

An analysis of the data secured can be made by referring to Table XII and figure 2. It will be seen that the soil temperature mean gradually rose until July 23 to 29, after which there was a gradual drop. At no time did the mean in the uncov red plot reach the inhibiting temperature (29° C.), but it closely approached this point during the warmest portion of the season. In the covered plot, however, the mean remained above 29° continuously until the cover was removed on August 3. The two important points to be considered in the respective plantings were (1) the amount of original infection which was determined three to four weeks after planting (see first and second observations in Table XII) and (2) the extent to which the disease either became systemic or was entirely outgrown by the plants during the following four or five weeks (see third observation in Table XII).

Considering first the amount of original infection, it will be seen that a high percentage of disease resulted in all the plantings of June 18 and June 26. The somewhat lower infection in that of June 18 may be explained in part at least by the fact that a smaller quantity of inoculum was used than in subsequent plantings. The next two plantings (July 10 and 19) were so made that the resulting seedlings were exposed during early growth to the maximum soil temperature of the season. By referring to Table XII it will be seen that associated with this higher temperature there was a decided reduction in the amount of infection in even the uncovered plots, while in the covered plots, where the mean temperature remained continuously above 29° C., no infection whatever occurred.

Considering, secondly, the extent to which the disease became systemic or was outgrown, it will be seen that in the planting of June 18 a majority of the infected plants showed systemic invasion at the second observation (thirty-ninth day). In the next planting (June 26), however, by the time of the second observation most of the external signs of the disease were confined to the dead cotyledons. The amount of systemic infection increased somewhat, however, at the third observation (39 per cent).

In the third planting (July 10) it is interesting to note first that the plants in the covered plot remained entirely free from infection. In the uncovered plot, although some cotyledon infection was noted at the second observation, no disease whatever was found at the third observation. This indicates that the time when the temperature was at its highest point the infected plants succeeded best in outgrowing the disease.

The field data secured in the foregoing experiments at Madison are thus in general accord with the experiments performed under controlled conditions. In such controlled experiments the amount of smut infection falls as the soil temperature rises toward 29° C. and is totally inhibited above this temperature. Likewise in the field trials with successive plantings there was a gradual reduction in the amount of infection following the rise in the mean soil temperature, with omplete inhibition of infection where the mean was kept above 29° for two or three weeks after planting. Complete freedom from infection under these Wisconsin field conditions was secured only by growing the plants under artificial conditions in which by covering the plants with glass the temperature was raised several degrees above the normal. It is, however, to be noted that the summer of 1920, when the foregoing results were secured, was somewhat cooler than the average. The weather records of other years indicate that in a hot summer complete inhibition of smut infection would be secured by such summer plantings.

CORRELATION OF EXPERIMENTAL RESULTS WITH FIELD CONDITIONS OF THE SOUTHERN STATES

These results obtained in both greenhouse and field experiments justify the question as to the part played by soil temperature in determining smut infection in the onion fields of the more southern States. As noted at the beginning of this article, a recent survey of southern onion sections indicates that smut is not prevalent in the southern fields—for example, in Texas—as it is in the northern onion sections. As was earlier explained, it is the practice in these southern fields to plant the onion seed in late summer or early autumn. It is thus quite possible that the mean temperature for the surface inch of soil in southern onion sections is considerably above the maximum for onion smut infection during and immediately following the sowing of seed. According to Mally (*6*), onion seed is sown in the Laredo district of southern Texas as early as August 1, while most of the seed is planted about September 10 to 25. The mean air temperature as recorded at Laredo, Tex., by the United States Weather Bureau for August, September, and October, 1917, is given in Table XIII.

TABLE XIII.—*Mean air temperatures for August, September, and October, 1917, at Laredo, Tex.*[a]

Day of month.	August.	September.	October.	Day of month.	August.	September.	October.
	°F.	°F.	°F.		°F.	°F.	°F.
1	89.5	85.5	71.0	17	90.0	79.0	80.5
2	88.5	86.0	80.0	18	90.5	83.0	81.5
3	88.5	86.5	80.5	19	91.0	75.5	76.0
4	88.5	87.0	80.5	20	92.0	79.5	67.5
5	89.0	88.0	80.5	21	90.0	79.5	62.0
6	88.5	87.5	78.0	22	90.0	80.0	63.5
7	89.0	88.0	81.0	23	90.0	79.5	68.5
8	88.5	88.5	82.5	24	91.0	79.0	61.0
9	89.0	89.5	64.5	25	89.5	80.5	66.5
10	90.5	88.0	64.5	26	87.0	81.5	76.5
11	90.5	83.5	70.5	27	89.5	81.5	74.5
12	88.0	85.0	73.5	28	86.5	71.5	78.5
13	89.5	86.5	75.0	29	89.5	71.5	75.0
14	88.5	88.5	77.0	30	84.5	71.5	50.5
15	90.5	83.5	76.0	31	86.0	.....	.....
16	88.5	83.5	80.0				

[a] Obtained by averaging the daily maximum and minimum temperatures.

Table XIII shows that the air temperature ranged very high during August and September, the onion-planting period. In this connection it is to be noted, moreover, that the records of Bouyoucos (*1*) in Michigan indicate that surface soil temperatures may considerably exceed air temperatures. Thus, his observations showed that the maximum temperature for the upper quarter inch of all the soils he studied was about 16° C. higher during hot, clear days than that of the air at an elevation

of 4 feet, while the minimum temperature of all the soils used, except peat, was 0.5° to 1.0° C. higher, as a monthly average, than that of the air. Our own observations in Wisconsin are in general accord with these Michigan records. Assuming that the temperature of the surface layer of soil under Texas conditions likewise averages several degrees higher than the air, it is evident that the mean never went below the point where infection is entirely inhibited (29° C., or 84° F.) during August and seldom below it during September. Continuing up to October 8 there were only a few days when the air temperature fell below 27.5° C. (81.5° F.), the point at which our experiments have shown smut infection to be markedly reduced. It seems probable, therefore, that even if onion smut were introduced into this Laredo soil, it would stand small chance of infecting onion seedlings to the extent of establishing the disease as a permanent factor. The data available are not sufficient to justify the attempt at more detailed geographic correlation of onion smut occurrence with the temperature factor. We believe, however, that the conclusion is justified that soil temperature during the early seedling stage must be considered as a limiting factor in determining the occurrence of the disease in any locality. It must be left with local observers to make use of this fact in interpreting conditions as they occur in any particular region.

SUMMARY

Onion smut was first noted in the Connecticut River Valley in 1869. Since then it has successively appeared and become an economic factor in nearly all of the northern onion-growing sections from New York to Oregon. This has probably resulted from chance introduction of the organism with seed or bottom sets, followed by its accumulation in the soil where continuous cropping with onions is practiced. The disease has not appeared in the southern onion-growing sections of Texas and Louisiana, although they are exposed to similar chance introduction of the parasite and the continuous cropping method is common.

These facts have raised the question as to wherein lies the explanation of the regional limitation of the disease. The southern method of culture, characterized by special seed bed and transplantation of seedlings, does not offer sufficient explanation for the absence of smut. No difference in susceptibility between northern and southern varieties has been found. Is regional limitation explained by differences in environmental factors in the North and the South at the time when the seedling is susceptible to infection, that is, during the first two or three weeks after germination? An analysis of certain of these factors in relation to infection has been the object of the present investigation.

The cotyledon of the onion is susceptible to attack by the smut organism up to the time it attains full growth, a period of about three weeks,

varying somewhat with environment. Cotyledons remaining free from infection during this period become resistant and serve as a barrier to subsequent invasion of the embryonic region of the true leaves. Consequently, if infection is prevented by environing conditions during this period of susceptibility, the plant will remain free during the remainder of its growth.

Experiments were conducted in which seedlings were grown on smut-infested soil held at various soil moisture contents. A high percentage of infected plants resulted over the entire range in which good germination and growth of the host occurred. At either extreme, very high or very low moisture, there was some reduction in amount of infection, but with it occurred a corresponding decrease in seed germination and rate of growth of the plants. Soil moisture, therefore, does not appear as a serious limiting factor in onion smut infection.

The relation of soil temperature to the development of the host and the parasite was studied by growing plants in pots held experimentally at a series of constant soil temperatures in the special apparatus known as the "Wisconsin soil temperature tank."

Seed germination and growth took place over a range of soil temperature from 10° to 31° C. Most rapid seed germination and development of tops occurred at soil temperatures of 20° to 25°, while as a rule the best development of roots occurred below 20°.

A high percentage of plants grown on smutted soil were infected at soil temperatures ranging from 10° to 25° C. A decided reduction in infection was noted at about 27°, and complete freedom from the disease resulted at 29°. In these experiments all plants were under uniform conditions of air temperature, which ranged from 15° to 20°.

The relation of variations in air temperature to the development of the disease was then studied.

Exposure of plants bearing incipient infections of the fungus in the aerial parts to an air and soil temperature of 30° to 33° C. so disturbed the relations between parasite and host as to preclude any further development of the disease. This was shown by growing plants at a temperature favorable for infection (15° to 20°). Then, just as the pustules of the disease were beginning to appear (tenth to twelfth day), the plants were removed to a room held at 30° to 33°. This stimulated top growth for a few days, which was followed by a decided checking of the plants and death after three or four weeks. However, if after 12 to 15 days at the high temperature the plants were returned to the original temperature (15° to 20°), they grew normally, but the fungus in nearly all cases failed to produce spores, and the plants remained free from further invasion.

Experiments were then performed in which seedlings were grown on infested soil held at 20°, 25°, and 30° C. with a uniform air temperature of 30° to 33°. A high percentage of infection resulted at soil temperatures

of 20° and 25°, but none at 30°, showing that high air temperature alone is insufficient to check the development of the disease. It appears probable that the failure of the fungus to complete its development in the case described above (where the plants after infection were exposed to an air and soil temperature of 30° to 33°) was brought about at least in part by some marked disturbance of the metabolism of the host and not simply by the direct effect of the high air temperature upon the fungus in the aerial parts of the seedling.

Comparison between the development of the disease in plants grown at 15° to 20° and at 24° to 28° C. (air and soil) was made. A high percentage of cotyledon infection occurred in both cases. At the lower temperature the disease proceeded as usual to the infection of the true leaves. At the higher temperature, however, the plants tended to outgrow the disease, this being associated with a more rapid rate of top development which apparently enabled the plants to slough off the smutted cotyledons before infection of the first true leaf occurred.

The foregoing conclusions as to the dominant influence of soil temperature upon onion smut infection, while primarily based on greenhouse experiments, have been found to accord well with field developments.

Successive out-of-door plantings at Madison, Wis., made in inoculated soil during the growing season, resulted in a gradual reduction of infection as the season advanced and the soil temperature rose. Complete freedom from smut was attained when the daily mean soil temperature at 1 to 2 inches depth remained at or slightly above 29° C. for two to three weeks. There was also a tendency, as the temperature rose, for the seedlings to outgrow the disease by the sloughing off of the diseased cotyledons before infection of the first leaf occurred.

An examination of records from one of the southern onion sections (Laredo, Tex.) shows that during a good share of the critical period for onion smut infection (August and September) the mean air temperature is above that at which complete inhibition of infection was attained in our experiments (29° C. or about 84° F.). If we assume, as observed in northern sections, that the mean temperature for the upper layer of soil is several degrees higher than that of the air at this time of the year, it is reasonable to conclude that even though the smut organism were introduced into southern onion sections, its development would be prevented or greatly minimized, first, by the prevention of infection due to high temperatures, and, secondly, by the rapidly developing tops outgrowing the disease, should occasional infections occur.

In general we believe, therefore, that the regional distribution of onion smut in the United States is conditioned upon the soil temperature during the seedling stage of the plant's growth, the infection and development of smut being favored by the relatively low temperatures and inhibited by the high temperatures, with approximately 29° C. as the critical point.

It is hoped that the evidence here recorded may lead to the accumulation of further field data bearing upon this particular problem by investigators in various places, especially in the southern States. It is also believed that these results illustrate well the importance of more persistent inquiry by the experimental method into the relation of environmental factors to the occurrence of disease of plants in general.

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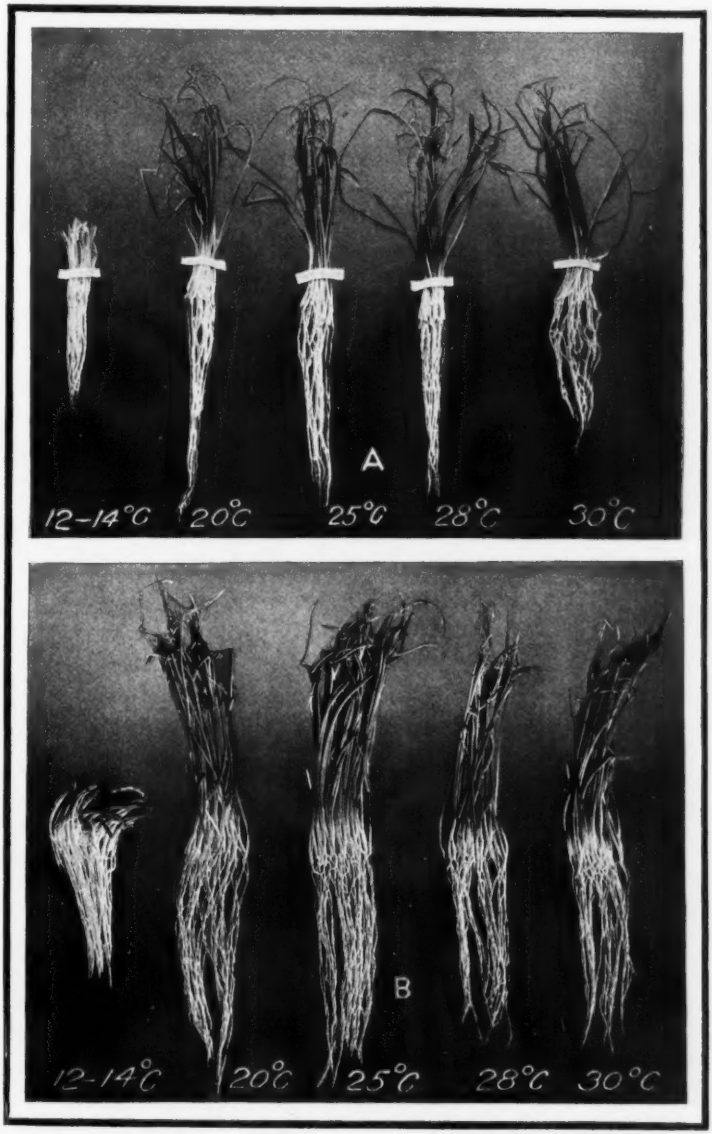
PLATE 25

Relation of soil temperature to the development of onion seedlings.

Upper row.—Seedlings of Red Globe variety 13 days old. Each cluster was the entire crop from one experimental culture pot. All were grown in like virgin soil and at the same air temperature (15° to 20° C.) but with gradation in the soil temperature of the respective pots as follows (left to right): 12° to 14°, 20°, 25°, 28°, 30°. Note that there is a tendency for greater root development in relation to top growth at the lower temperatures. This was especially marked at the lowest temperature, 12° to 14°. For further details see Table V and the accompanying text.

Lower row.—Seedlings of Yellow Bermuda variety grown under same conditions as those in upper row.


A
12-14°C
20°C
25°C
28°C
30°C
B
12-14°C
20°C
25°C
28°C
30°C

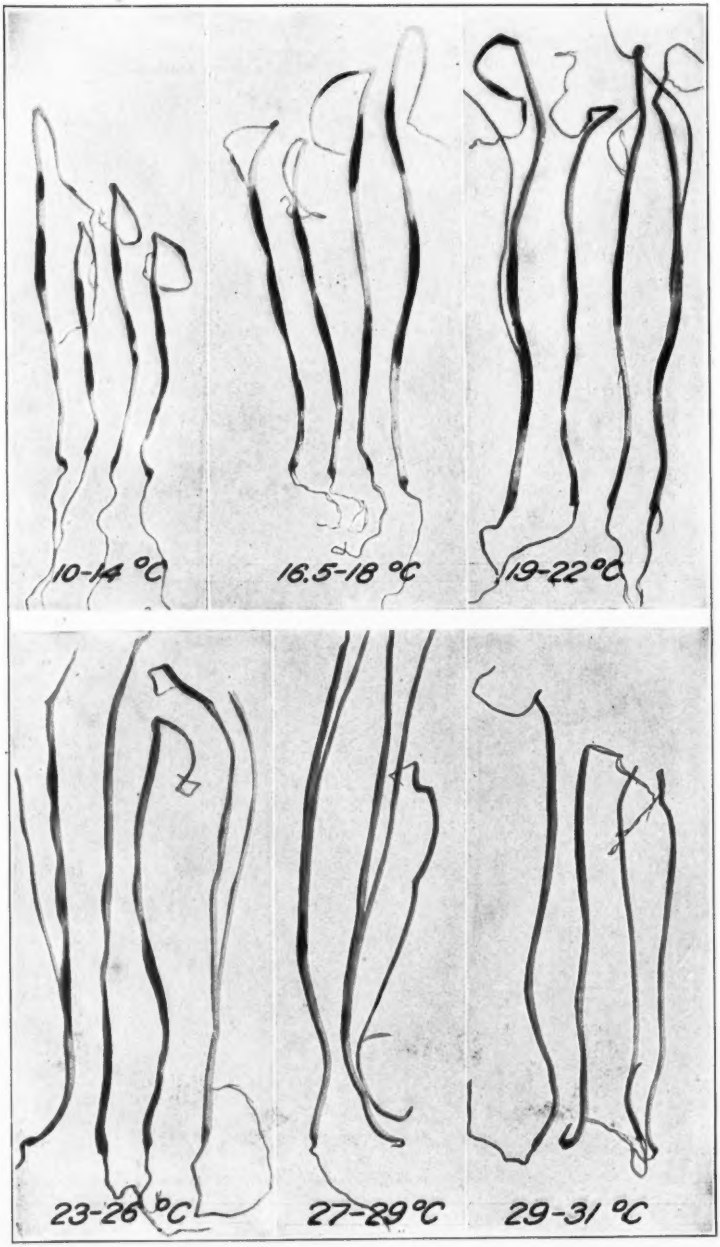

10-14 °C
16.5-18 °C
19-22 °C
23-26 °C
27-29 °C
29-31 °C

PLATE 26

Relation of soil temperature to the infection of onion seedlings by the smut fungus, *Urocystis cepulae*.

Representative seedlings taken from the experimental culture pots, showing the influence of soil temperature upon the amount of smut. All the pots alike contained smut-infested soil. The air temperature and other aerial factors were the same for all. Soil temperature was the only factor varied experimentally, the temperature gradations extending from about 10° to 30° C.

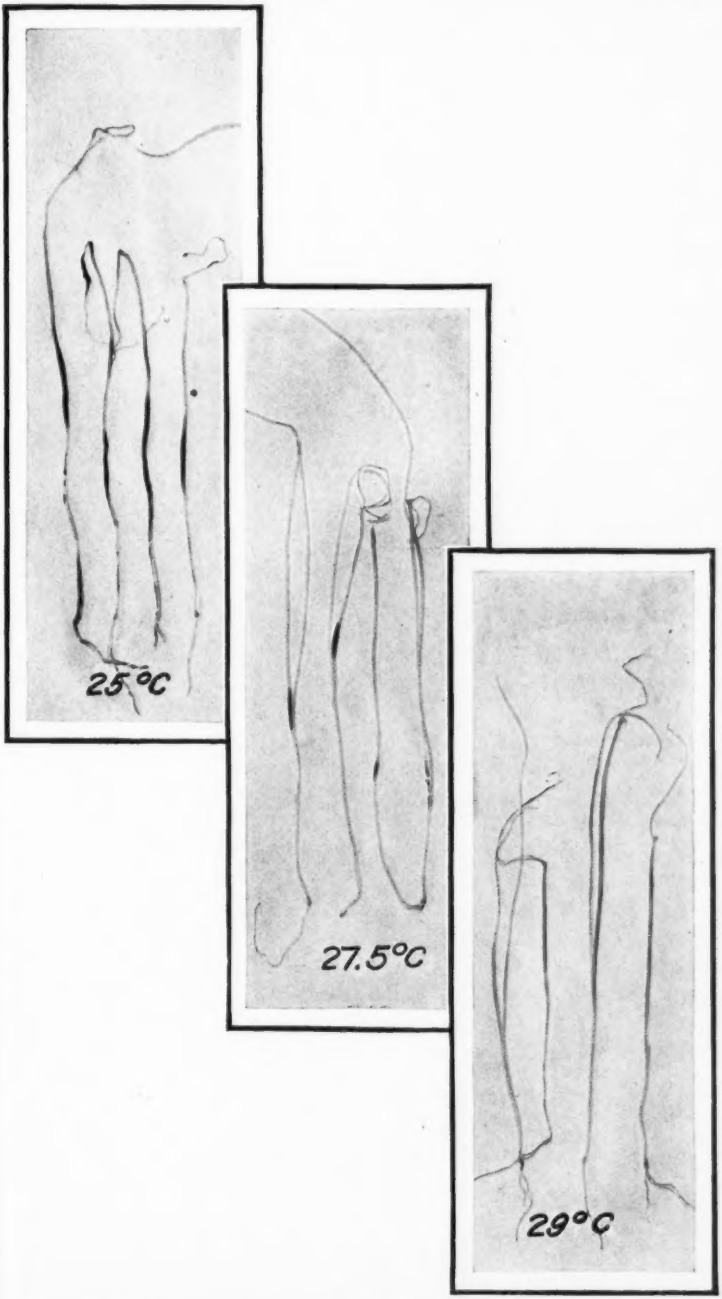
Note the abundance of smut at 10° to 22°, as shown in the upper row. A slight reduction occurred at 23° to 26°. At 27° to 29° the reduction is sharply marked. At 29° to 31° inhibition is complete. For the percentages of seedling infection and other details see Table VI and the accompanying text.

PLATE 27

Relation of soil temperature to onion smut infection.

This shows the results from a series of experiments in which the methods described for Plate 26 were repeated with the soil temperatures restricted to the critical limits between 25° and 29° C. and controlled more exactly. Note the marked reduction in infection at 27.5° and complete inhibition at 29°, thus establishing, but with more exactness, the conclusions illustrated in Plate 26.

For the percentage of infected seedlings at these temperatures and other details, see Table VIII and the accompanying text.


25°C
27.5°C
29°C

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A PHYSIOLOGICAL STUDY OF GRAPEFRUIT RIPENING AND STORAGE [1]

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In an earlier investigation (7) [2] the changes in Florida-grown grapefruit during storage were studied, particular attention being paid to the sugar and acid content of the pulp or edible portion of the fruit as influenced by some six different storage temperatures. It was found that the acid content decreased in cold storage while the total sugar content remained about the same. The percentage of cane sugar decreased and the reducing sugar content increased. At the higher temperatures, common storage (about 55° to 60°, 70°, and 86° F.) there was in some cases apparently an increase in acidity and a reduction in the amount of sugar, especially in fruit stored for long periods. The shrinkage, which was very marked in the ventilated packages at these high temperatures, made the obtaining of definite evidence on this point impossible.

The investigation described in the present paper is concerned with the acid and sugar changes in the fruit on the tree as well as with the changes which take place, both in warm storage and in cold storage, in fruit picked at monthly intervals. The control of the pitting which occurs commonly on grapefruit in cold storage is given some attention.

PLAN OF THE EXPERIMENTS

"Common Florida" [3] fruit from two trees was picked at monthly intervals for four months, beginning July 27, making five different picks. At the last three pickings fruit was also harvested from two additional trees in the same grove. The fruit was expressed to Washington and sampled on arrival. Part of the fruit was placed in warm storage at about 70° F. and part in cold storage at 32°. Analyses were made of the fruit stored in the warm storage each month for two months and on fruit from the last three pickings stored in cold storage, after it had been in storage four months. By this plan it was possible to obtain data on the changes in the fruit on the tree from a month or so before the fruit was in condition to pick for market until the last of the season, and to compare the changes which took place in cold storage in fruit picked from the same trees at different times of the season.

[1] This paper gives the results of a portion of the work carried on under the project "Factors affecting the storage life of fruits."

[2] Reference is made by number (italic) to "Literature cited," p. 278–279.

[3] The writer's thanks are due Mr. W. J. Krome for the picking and shipping of all the "Common Florida" fruits used in these experiments.

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METHODS OF ANALYSES

The fruit was prepared for sampling and sampled as in the previous work. Analyses were made for acids, sugars, both reducing sugars and total sugars, dry weight, shrinkage, thickness of peel, and percentage of peel. In addition the acidity and specific gravity of the expressed juice of the fruits were determined and the solids-acid ratio calculated after the usual method. The acidity determinations were carried out as in the previous work, as were practically all the other determinations with the exception of the extraction of the sugar from the pulp. In the sugar extraction a method was followed similar to that described in work on potatoes (*6*). The weighed pulp was placed in a liter volumetric flask which was then filled to volume with 85 per cent alcohol. It was allowed to stand with frequent shakings for about three weeks, the losses from evaporation, of course, being made up by adding alcohol. The alcoholic solution of sugar was then separated from the residue by filtration, and the sugars were determined in aliquots of the filtrate.

The first lot of grapefruit was of small size, green in color, with very little juice in the pulp. No solids-acid determinations were made on this lot. They were, however, maintained in warm storage for two months. At the end of this period many of them had assumed the characteristic yellow color of the ripe grapefruit.

The second pick, received August 29, was much further advanced, being about 50 per cent colored and of good size. The third and fourth picks, those of October 25 and November 28, respectively, were in fine condition for shipping and are what would be considered midseason fruit. The November 28 fruit was fair, possibly a little coarser than the two picks immediately preceding. No sprouted seeds were found in any of the fruits, however. The date of picking might be considered as in the latter part of the grapefruit season for this locality and for this variety.

The analyses of grapefruit picked from trees 1 and 2, from warm storage at about 70° F. for one and two months, are shown in Tables I and II. In the analytical work the analyses were usually made in duplicate, and both analyses are given in the tables, as this furnishes evidence on the experimental error in the method of sampling. The tables are self-explanatory.

An inspection of Tables I and II shows that in the first four pickings there is in all cases an increase in the acid content of the pulp, while in the last picking from both trees there is no decided increase. A comparison of the acid content, as determined in the analyses of the pulp and the acid content of juice, shows a similar behavior. The acid content of the juice is, as a rule, markedly higher than that of the pulp, due, of course, to the fact that in the last-mentioned case the weight of fibrous material is taken into consideration in calculating the percentage

of acid. In the fifth pick from both trees there is no decided variation in the acid content of the pulp during storage, and the percentage of acid in the juice does not change as much as in fruit from any of the other four picks. With the sugars, the percentage of reducing sugars and of total sugars is always greater at the end of two months in warm storage, except in the fifth pick. The reducing sugar increases most, due probably to the inversion of some of the cane sugar which is less in all cases after two months in storage.

It was brought out in the earlier publication on grapefruit storage that there was an indication that the acid content of the fruit was slightly increased during a long period of warm storage. It was pointed out also that definite evidence on this point was difficult to obtain because the structure of the fruit prevented accurate calculation of the shrinkage of the various portions. Further evidence, mostly of an indirect nature, may be derived from the data on sugar and acid content of the fruit, found in Tables I and II. As was mentioned above, there is in all cases an apparent increase in the acid content and the total sugar content of the pulp, due for the most part undoubtedly to loss of water during storage. In the tables it is noticeable that the solids-acid ratio is usually less after two months in storage. This indicates, of course, that the increase in soluble solids is not proportional to the increase in acidity and that some soluble substance or substances other than titratable acids decreased in the storage period. This occurs in five cases out of seven on which data were obtained. The other two cases, tree 2, third pick and fifth pick, show slight increases, 0.07 and 0.11, respectively.

These data are corroborated in the total sugars-acid ratios, which are calculated by dividing the percentage of total sugar as dextrose by the percentage of acid as citric. In the 10 cases the ratio of sugar to acid is less in 7, practically the same in 2, and greater in 1. Indications are, then, that there is usually an increase in the ratio of sugar to acid under the conditions of the experiment. This could be brought about by either decreasing the sugar content of the fruit or by increasing the acid content or by a combination of these two factors. It is noticeable that in 6 cases out of 10 the acid-sugar ratio is greater after one month in storage than it is after two months at the same temperature. The acid and sugar in the fruit from warm storage will be considered later in comparison with the changes taking place in cold storage.

There is in most cases not much variation in the percentage of dry matter during storage, though there seems to be a tendency, more marked in some cases than in others, toward an increase. This seems probable, as the shrinkage where determined is from 14.4 to 23.3 per cent for the full two months in storage. The percentage of peel always decreases during storage at this temperature, due to the loss of water and wilting. This is evident in the decrease in thickness of the peel, which is very marked, especially in the earlier picks.

CHANGES IN FRUIT ON TREES

The analyses of fruit from the various pickings at the time it was placed in storage (Tables I and II) show marked differences in composition.

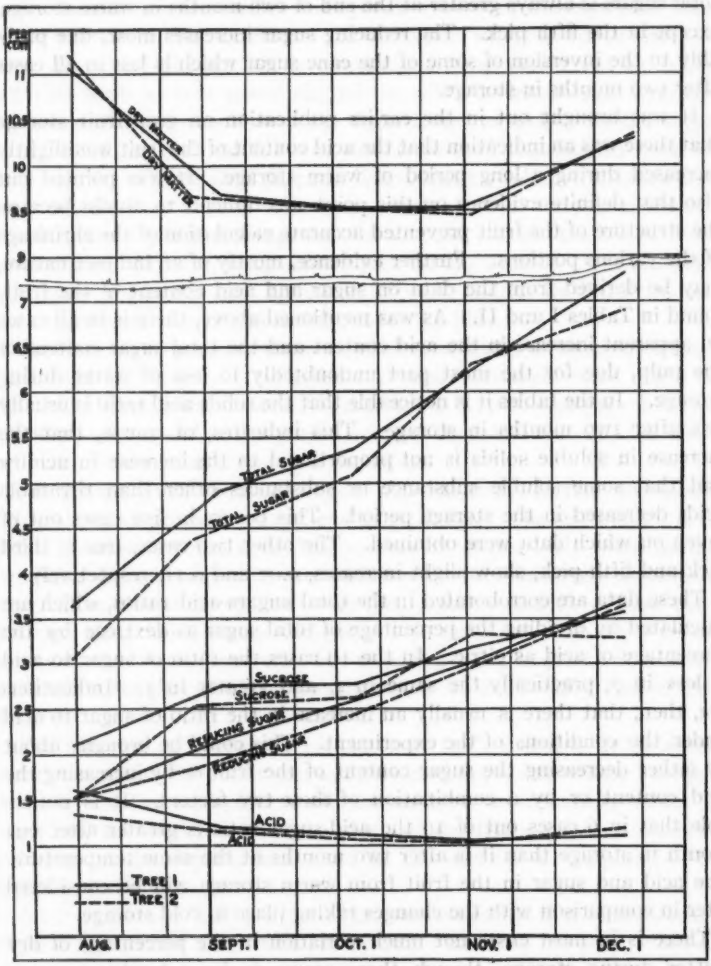

FIG. 1.—Graphs showing changes in percentage of dry matter, acid, and sugars calculated as dextrose in fruit on two Common Florida trees from August to December.

For convenience in comparison the data for sugars, acids, and dry matter are shown graphically (fig. 1). In these curves the percentage is plotted on the ordinates and the time interval between pickings on the abscissae.

TABLE I.—*Percentage of acid, sugar, and dry matter in pulp, thickness of peel and percentage of peel, acid and soluble solids in juice, and solids-acid ratio of "Common Florida" grapefruit from tree No. 1, picked at monthly intervals and stored at 70° F.*

	First pick.			Second pick.			Third pick.			Fourth pick.			Fifth pick.		
	Placed in storage July 29.	Second sampling Sept. 3.	Third sampling Oct. 8.	Placed in storage Sept. 3.	Second sampling Oct. 8.	Third sampling Nov. 3.	Placed in storage Oct. 4.	Second sampling Nov. 4.	Third sampling Dec. 4.	Placed in storage Oct. 30.	Second sampling Dec. 5.	Third sampling Jan. 3.	Placed in storage Dec. 5.	Second sampling Jan. 6.	Third sampling Feb. 6.
Acid as percentage of citric	1.61 1.49	1.63 1.67	1.81 1.76	1.14 1.26	1.45 1.28	1.34 1.38	1.10 1.08	1.21 1.23	1.18 1.23	1.00 1.02	1.18 1.17	1.18 1.21	1.14	1.13 1.17	1.17
Percentage of reducing sugar as dextrose	1.59 1.46	1.96	2.99 3.00	2.17 2.19	2.91 2.90	3.27 3.38	2.62 2.58	3.05 2.86	3.48 3.40	2.96 3.04	4.71	4.09 4.07	3.73	4.43 4.39	4.48 4.57
Percentage of cane sugar as dextrose	2.08 2.18	2.97	1.20 1.25	2.62 2.88	2.57 2.40	2.08 2.25	2.61 2.93	2.86 2.68	2.48 2.49	3.13 3.22	2.31	2.79 2.90	3.57	3.56 3.20	2.90 2.60
Percentage of total sugar as dextrose	3.67 3.64	4.93	4.19 4.25	4.79 5.07	5.48 5.30	5.35 5.63	5.23 5.51	5.91 5.54	5.96 5.89	6.09 6.26	7.02	6.88 6.57	7.30	7.99 7.59	7.38 7.17
Total sugar-acid ratio	2.35	2.98	2.35	4.10	3.96	4.03	4.92	4.68	4.93	6.1	6.0	5.63	6.4	6.77	6.21
Percentage of dry matter	11.20 11.38	11.44 11.30	11.27	9.75 9.59	9.72	9.56 9.63	9.49 9.63	9.28 9.13	9.66 9.62	9.45	10.24	10.10	10.32 10.35	.....	10.32 10.81
Percentage of peel	33.3	32.3	26.1	25.6	23.2	22.7	23.3	19.9	19.8	21.8	.....	16.7	21.2	14.3	15.8
Thickness of peel	6.8	5.2	5.0	6.1	4.8	4.9	5.5	3.8	3.9	5.0	3.0	2.6	4.5	2.2	2.
Percentage of shrinkage	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	14.4	14.4	.....	.....	23.
Acidity of juice as percentage of citric acid	.....	.....	.....	1.44	1.56	1.65	1.28	1.11	1.35	1.14	1.45	1.46	.....	1.4	1.3
Soluble solids (Brix)	.....	.....	.....	9.21	10.29	9.99	9.45	10.61	9.81	10.11	11.52	11.47	.....	11.81	11.47
Solids-acid ratio	.....	.....	.....	6.36	6.61	6.05	7.38	9.50	7.23	8.81	7.93	7.87	.....	8.45	8.80

TABLE II.—*Percentage of acid, sugar, and dry matter in pulp, thickness of peel and percentage of peel, acid and soluble solids in juice, and solids-acid ratio of "Common Florida" grapefruit from tree No. 2, picked at monthly intervals and stored at 70° F.*

	First pick.			Second pick.			Third pick.			Fourth pick.			Fifth pick.		
	Placed in storage July 29.	Second sampling Sept. 4.	Third sampling Oct. 5.	Placed in storage Sept. 3.	Second sampling Oct. 7.	Third sampling Nov. 3.	Placed in storage Oct. 4.	Second sampling Nov. 4.	Third sampling Dec. 4.	Placed in storage Nov. 1.	Second sampling Dec. 8.	Third sampling Jan. 5.	Placed in storage Dec. 9.	Second sampling Jan. 5.	Third sampling Feb. 6.
Acid as percentage of citric	1.35	1.51	1.63	1.16	1.24	1.56	1.06	1.14	1.25	1.06	1.16	1.23	1.15	1.12	1.21
		1.57	1.65	1.2	1.29	1.5			1.26	.98	1.17			1.13	
Percentage of reducing sugar as dextrose	1.48	2.51	2.79	1.78	2.93	3.45	2.47	2.68	3.48	3.01	3.59	4.26	3.73	4.09	4.39
	1.56	2.16		1.92		3.18	2.44		3.40	2.92	3.51	4.46	3.66		4.35
Percentage of cane sugar as dextrose	1.52	2.04	1.43	2.52	2.11	2.06	2.74	2.29	2.16	3.30	2.73	2.79	3.24	4.65	2.86
	1.54	2.28		2.54		2.36	2.52		2.03	3.38	2.89	2.68	3.25		2.78
Percentage of total sugar as dextrose	3.00	4.55	4.22	4.30	5.04	5.51	5.21	4.97	5.64	6.31	6.32	7.05	6.97	8.74	7.25
	3.10	4.44		4.46		5.54	4.96		5.43	6.30	6.40	7.14	6.91		7.13
Total sugar-acid ratio	2.25	2.91	2.57	3.71	4.0	3.6	4.79	4.35	4.42	6.17	5.48	5.76	6.03	7.80	5.94
Percentage of dry matter	11.13	11.24	11.22	9.72	9.60	10.53	9.54	9.80	9.55	9.66	10.36	11.16	10.30	10.09	10.18
	11.05	11.46		9.59		10.46				9.43	10.23	11.15			
Percentage of peel	45.8	34.8	28	29.1	24.2	24.1	23.5	23.8	20.4	21.2	21.8	13.2	18.2	18.7	14.7
Thickness of peel	8.1	7.7	5.2	6.9	4.6	5.0	5.6	5.3	4.3	4.2	3.8	2.9	3.2	3.0	2.3
Percentage of shrinkage	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	19.6	19.5	.....	10.1	18.0
Acidity of juice as percentage of citric	.....	.....	.....	1.44	1.59	1.68	1.29	1.41	1.5	1.18	1.32	1.51	1.19	1.28	1.28
Soluble solids in juice (Brix)	.....	.....	.....	9.27	10.6	9.92	8.15	9.55	9.59	10.33	10.59	10.97	10.79	10.77	11.67
Solids-acid ratio	.....	.....	.....	6.41	6.63	5.88	6.31	6.74	6.39	8.73	7.97	7.26	9.03	8.41	9.14

From an inspection of the curves it is evident that there is a decrease in acidity as the season advances, the acid being highest in both cases at the beginning of the season. The acid content is lowest at the fourth pick and rises slightly at the fifth pick. Collinson (*3*) shows a somewhat similar decrease in acidity. This writer analyzed the fruit at more frequent intervals but apparently did not begin sampling so early in the season. According to his work there is a general tendency toward lower acidity, though in a few of his series of analyses there is a higher acidity toward the end of the season than in the midseason fruit.

There is a rise in the percentage of total sugars during the season, the total sugar content of pulp of the fifth pick being about double that of the first pick. Collinson shows an increase in the total sugar content, but it is not so marked, due probably to the fact that his series begins later in the season. As shown in the curves (fig. 1), the rise in total sugar content during the first month is very sharp. The increase in percentage of reducing sugar during the season is much more gradual and regular than that of the total sugars. The percentage of this sugar in the pulp a little more than doubles in the four months of the experiment. Much the same ratio of increase is found in the total sugars. The cane sugar curves are not so regular. There is, however, a marked increase in the percentage of cane sugar. The mean of the two sucrose curves is always higher than that of the reducing sugars except at the last sampling. Collinson records a series of analyses in which the reducing sugar is markedly higher than the sucrose during the latter part of the season. The irregularities in the total sugar curves in the present work are due to the variation in sucrose content.

The percentage of dry matter, as determined in this work, is highest at the first of the season between 11 and 11.5 per cent but drops in the first month to between 9.5 and 10 per cent, the third and fourth analyses giving about the same results. There seems to be, however, an increase in the dry weights in the last month.

A comparison of the percentage of peel (Tables I and II) at the time the five different lots of fruit were placed in storage shows there is a decrease from 33.3 to 21.2 and 45.6 to 18.2 per cent of peel by weight for trees No. 1 and 2, respectively. The percentage of peel decreases much more rapidly in the first month than in the succeeding month. In fact, in the case of tree 1 there is only a slight decrease in the proportion of peel to pulp in the last three pickings. At the time these last three pickings were made, the fruit was ready for market.

As might be expected, the decrease in thickness of the peel, as measured in these experiments, parallels the decrease in percentage of peel. The peel was found to be 6.8 mm. and 8.1 mm. thick, respectively, for trees 1 and 2 when the first pick was placed in storage, while it measured 4.5 mm. and 3.2 mm. at the first sampling of the fifth pick. This is a reduction of 34 per cent and 60 per cent in the thickness of the skin for

the season. It is evident that the proportion of peel to pulp and thickness of the peel decrease as the fruit matures.

A comparison of the acid and sugar changes in grapefruit in growth and ripening with the acid and sugar changes of other fruits in the same period of their life history brings out some interesting correlations and differences. The total sugar content of deciduous fruits usually increases during the growing and ripening period. This has been shown for apples by Lindet, (*8*) Bigelow, Gore, and Howard (*2*), and others; for pears by Ritter (*12*), Riviere and Bailhache (*11*), Magness (*10*), and by Bigelow and Gore (*1*), for peaches. Numerous other investigations corroborating this point might be mentioned. The literature pertinent to this subject may be found in the works referred to here. With certain vegetables a somewhat similar increase in total sugars is found. This was brought out by Hasselbring (*5*), working with sweet potatoes, and Sando (*14*) with tomatoes. This evidence would seem to indicate that in fruits or vegetables where sugar is stored the percentage of sugar calculated on a wet-weight basis increases during the growing season—that is, there is not only an absolute increase but an increase in proportion of sugar present as compared to the sum of the other constituents. This increase in some cases is due to an increased content of reducing sugars, as in the tomato, or may be due to an increase in both reducing sugars and cane sugar, as in the apple, pear, and peach, or for the most part to an increase in cane sugar, as in the sweet potato.

In regard to the acid content of fruits which contain both sugar and acids in appreciable quantities, there is sometimes an increase and sometimes a decrease in acidity as the season advances. In pears there is generally a decrease. Magness (*10*), however, found that pears from the Yakima district, Washington, and Medford district, Oregon, showed an increased acidity as the season advanced. Apples, according to the analyses of Lindet (*8*), and Bigelow, Gore, and Howard (*2*), exhibit a decrease in acidity as the growing season advances. Peaches, on the other hand, increase in acid content as they approach maturity. The decrease in acidity of grapefruit during the growing season is comparable to the usual behavior of the acidity in pears and in apples.

COLD STORAGE EXPERIMENTS

As mentioned earlier in this article, experiments on the cold storing of grapefruit were carried out during the 1920–21 season. Fruit from four trees was used. These trees included the two from which fruit was obtained for the warm storage work, the fruit being from the lots designated third, fourth, and fifth picks in the experiments already described. Not sufficient fruit was available for this work from the fifth pick from tree 1, so only two experiments were possible with fruit from this tree.

Table III gives the results of analyses made at the time the fruit was placed in storage and four months later. As was pointed out, because of the structure of the fruit it is very difficult to obtain definite evidence on the changes of the various constituents of the pulp. While the

fruit for each experiment was carefully selected from a lot of fruit all harvested at the same time from a single tree, the variation in composition of the fruit on this tree introduces a possible error which it is hardly possible to calculate. It is only by obtaining a large amount of evidence that a clear indication of the direction of the change can be obtained. It was accordingly deemed advisable to give in this table all the data obtained in the analyses in the 11 different storage experiments carried out in this portion of the investigation. The table is self-explanatory.

TABLE III.—*Percentage of acid, sugar, and dry matter in pulp, thickness of peel and percentage of peel, acid and soluble solids in juice, and solids-acid ratio of "Common Florida" grapefruit before and after storing four months at 32° F.*

TREE 1

	First lot.		Second lot.		Third lot.	
	When placed in storage.	After 4 months in storage.	When placed in storage.	After 4 months in storage.	When placed in storage.	After 4 months in storage.
Acid as percentage of citric	1.10	0.88	1.00	0.88	.....	.....
	1.08	.90	1.02	.92	.....	.....
Percentage of reducing sugar as dextrose	2.62	2.55	2.96	3.48	.....	.....
	2.58	2.74	3.04	3.09	.....	.....
Percentage of cane sugar as dextrose	2.61	3.11	3.13	3.11	.....	.....
	2.93	2.72	3.22	3.51	.....	.....
Percentage of total sugar as dextrose	5.23	5.66	6.09	6.59	.....	.....
	5.51	5.46	6.26	6.6	.....	.....
Percentage of dry matter	9.49	9.43	9.45	9.74	.....	.....
	9.63	9.37	9.54	9.74	.....	.....
Percentage of peel	23.3	26.9	21.8	21.6	.....	.....
Thickness of peel (in mm.)	5.5	5.5	5	4.3	.....	.....
Percentage of shrinkage	.....	5	.....	4.9	.....	.....
Acidity of juice as percentage of citric	1.27	1.02	1.14	1.01	.....	.....
Soluble solids (Brix)	9.45	8.87	10.11	11.25	.....	.....
Solids-acid ratio	7.38	8.66	8.81	11.16	.....	.....

TREE 2

Acid as percentage of citric	1.06	0.98	1.06	1.00	1.15	1.10
Percentage of reducing sugar as dextrose	2.47	2.61	3.01	3.37	3.73	3.29
	2.44		2.92	3.54	3.66	
Percentage of cane sugar as dextrose	2.74	2.65	3.30	3.03	3.24	3.59
	2.52		3.38	2.75	3.25	
Percentage of total sugar as dextrose	5.21	5.26	6.31	6.40	6.97	6.88
	4.96		6.30	6.29	6.91	
Percentage of dry matter	9.54	9.46	9.66	9.86	10.30	9.77
			9.43	10.16		9.82
Percentage of peel	23.5	23.3	21.2	21.7	18.2	22.4
Thickness of peel (in mm.)	5.6	4.4	4.2	4.3	3.2	4.8
Percentage of shrinkage	.....	3	.....	4	.....	3.9
Acidity of juice as percentage of citric	1.28	1.19	1.18	1.09	1.19	1.05
Soluble solids (Brix)	9.40	10.04	10.33	10.16	10.79	11.35
Solids-acid ratio	7.31	8.44	8.73	10.29	9.03	10.84

TABLE III.—*Percentage of acid, sugar, and dry matter in pulp, thickness of peel and percentage of peel, acid and soluble solids in juice, and solids-acid ratio of "Common Florida" grapefruit before and after storing four months at 32° F.*—Continued

TREE 3

	First lot.		Second lot.		Third lot.	
	When placed in storage.	After 4 months in storage.	When placed in storage.	After 4 months in storage.	When placed in storage.	After 4 months in storage.
Acid as percentage of citric	1.06	0.98	1.10 1.09	1.03 1.07	1.29 1.18	1.12 1.13
Percentage of reducing sugar as dextrose	2.46 2.58	2.55 2.35	2.77 2.50	3.22 3.50	3.36 3.51	3.31 3.51
Percentage of cane sugar as dextrose	2.83 3.01	2.58 2.77	2.81 2.81	3.16 2.75	3.01 3.25	2.86 2.88
Percentage of total sugar as dextrose	5.29 5.59	5.13 5.12	5.58 5.31	6.38 6.25	6.37 6.76	6.17 6.39
Percentage of dry matter	8.77 8.78	8.10 8.53	9.56 9.16	9.19 9.67	9.82 9.68	10.12 10.26
Percentage of peel	24	24.2	22.2	21.8	22.2	20.3
Thickness of peel (in mm.)	5.3	4.7	4.9	4	4.3	4
Percentage of shrinkage	.....	5	.....	4.3	.....	4.6
Acidity of juice as percentage of citric	1.26	1.17	1.25	1.08	1.29	1.17
Soluble solids (Brix)	8.65	11.19	9.66	9.46	10.29	11.19
Solids-acid ratio	6.92	9.51	7.71	8.73	7.94	9.51

TREE 4

Acid as percentage of citric	1.18 1.10	0.94 1.03	1.24	1.04	1.21 1.22	1.06 1.18
Percentage of reducing sugar as dextrose	2.70 2.66	2.18 2.80	3.04	3.21 3.23	3.75 3.85	3.28 3.30
Percentage of cane sugar as dextrose	2.62 2.56	3.31 2.69	1.78	3.03 3.16	3.10 3.02	2.78 2.49
Percentage of total sugar as dextrose	5.32 5.22	5.49 5.49	5.82	6.24 6.39	6.85 6.87	6.06 5.79
Percentage of dry matter	9.28 9.53	9.91	9.58 9.57	9.97 9.96	9.87 9.90	9.79 9.94
Percentage of peel	22	26.4	22.3	21.7	21	24.5
Thickness of peel (in mm.)	5.4	5.3	4.5	4.9	4	5.3
Percentage of shrinkage	.....	4.9	.....	3.3	.....	4
Acidity of juice as percentage of citric	1.36	1.17	1.31	1.04	1.26	1.18
Soluble solids (Brix)	9.35	10.01	10.23	10.16	10.86	10.59
Solids-acid ratio	6.86	8.55	7.79	9.73	8.60	8.90

From Table III it is evident that in every case there is a lower acidity in the fruit after it has been held in storage four months than in fruits from the same tree and picking when placed in storage. This is in accordance with the findings reported in the previous publication and would seem to establish this point definitely. The fact that in the present experiments fruit was picked at three different times during the growing and ripening season strengthens the evidence.

The total sugar content is usually slightly higher at the end of the four months' storage period, though there are several instances in which it is lower. These cases are mostly in well-matured fruit of the last pick. The increase in total sugars is due for the most part to an increase in the reducing-sugar content, as there is usually a marked decrease in the percentage of cane sugar during storage. There is never more than 5 per cent shrinkage during these four months. This shrinkage is doubtless partly from the peel and partly from the interior portion or pulp.

The fact that in most cases there is an apparent increase in total sugars can be accounted for by the loss of water and consequent shrinkage. It is very evident from these data that there is no appreciable diminution in the amount of sugar in the grapefruits in four months at 32° F. On the other hand, there is without doubt no considerable increase. It is, of course, probable that some of the pectins and other hemicelluloses or the glucosid in the fruit break down slowly, and it is possible that some reducing substance is formed from these decomposition products.

A comparison of the behavior of the acids and sugars in grapefruits stored in warm storage (Tables I and II) with the results obtained in the cold storage experiments just considered brings out some rather striking differences. In the data obtained from the warm storage experiments there is evidence of an increase in acidity or a decrease in total sugars or both—that is, in most cases the ratio of total sugar to acid decreases, while in the cold storage the reverse is true. This is corroborated by the acidity and soluble solids of the juice. In the warm storage experiments the solids-acid ratio is in most cases less after two months in storage, while in the cold storage there is always a decrease in acidity and an increase in solids-acid ratio. It is evident that there is an increase, or at least not a decrease, in acidity in warm storage and a decided decrease in cold storage. It would, therefore, seem probable that some of the processes which go on in the fruit stored in the warm are modified when the fruit is placed in cold storage. It is possible, of course, that in respiration the acid is used up in cold storage while the sugars are used in warm storage. There is an indication that the sugar content may decrease slightly in the fruits held in warm storage, while there is no evidence of change in the percentage of sugar in the cold-stored fruits. Magness (*9*) has shown that the composition of the gases in the interior of apples and potatoes varies with the temperature at which they are held. For example, he found that the gas from the interior of Yellow Newtown apples stored at 2° C. (about 35° F.) analyzed 14.2 per cent O_2 and 6.7 per cent CO_2, while at 30° C. (86° F.) the extracted gas was 3.2 per cent O_2 and 21.4 per cent CO_2. The air surrounding the fruits used in these experiments was practically the same in both cases. The oxygen content was low and the carbon-dioxid content high in the fruit at high temperatures because the oxygen was used up in respiration faster than it could diffuse in from the outside. While no such determinations have been made on grapefruits, it seems

probable from the size of the fruit and the thickness and structure of the peel that in fruit held at high temperatures for any considerable period there would be a low oxygen pressure. This might result in some intermolecular respiration and the formation of acid. At low temperatures the respiration rate would be considerably decreased, while the rate of diffusion of O_2 through the tissues would not be so greatly reduced, and sufficient oxygen might be present for the breaking down of the compounds used in respiration of CO_2 and H_2O. A careful investigation of this point is needed. The work of Gerber (*4*) is of interest in this connection.

The dry weights are about the same at the conclusion of the experiments as at the beginning. There may be a slight diminution in the percentage of dry matter, but this apparently lies within the experimental error of the determinations. The variation in thickness of peel and percentage of peel is so great that there is frequently a higher percentage of peel after the fruit has been stored four months than when it was placed in storage. This is undoubtedly due to the lack of uniformity in the fruits and the low percentage of shrinkage.

The loss in weight during the four months' storage is from 3 to 4.9 per cent, averaging around 4 per cent. The relative humidity of the storage rooms was around 75 per cent. The fruit was not in the best condition for merchandising at the end of this storage period, as it was in many cases badly pitted. It is doubtful whether this method of storage would be applicable to commercial conditions if the fruit were placed directly in cold storage.

EXPERIMENTS IN THE CONTROL OF PITTING

As was mentioned in the earlier paper (*7*), grapefruit tends to pit in cold storage. This pitting begins as a small indentation of the skin in practically any region of the surface. The sunken area gradually increases in size, frequently becoming as much as 1 cm. in diameter. They are usually, in the type of fruit used in these experiments, about 1 mm. in depth. In time they may take on a brown color. This coloring occurs more quickly if the fruit is removed to a warm room. These pits may be very numerous on the surface of the fruit, in many cases coalescing in irregular shaped patches.

Cross sections of these pits show that they are formed by a breaking down of the layer of tissue containing the oil vesicles. There is apparently no disintegration of the tissue. The cells and vesicles simply flatten out as if subjected to local pressure, the layer of tissue becoming brown. The injury apparently does not extend to any distance in the spongy tissue beneath this oil-bearing layer, and it is only after a long period that any evidence of the discoloration appears on the inside of the peel. The pulp of the fruit is apparently uninjured. The affected fruit, however, is very unsightly, and badly pitted fruit would hardly be salable in a normal market. It was evident that unless some method of preventing

this pitting was worked out the storing of grapefruit for any considerable period would hardly be commercially practicable.

Experiments were, therefore, undertaken to see if it were possible to treat or handle the fruit so that it could be cold-stored without this danger of pitting. As was mentioned earlier, fruit stored in warm storage, 70° to 86° F., or in common storage (about 55° to 60°) apparently does not pit. It was considered possible that if fruit were cured for a time in warm storage before being placed in cold storage the injury from this blotching and breaking down of the surface of the peel might be obviated. Accordingly a lot of 1 dozen fruits from tree 1, third pick, was maintained at a temperature of 70° and a humidity of about 65 per cent for one month, then removed to cold storage (32°) and xamined at intervals. At the end of three months in cold storage none of these fruits were pitted, while about 60 per cent of the fruit from the same lot placed directly in cold storage at 32° were badly pitted.

The experiment was repeated with grapefruits of the Duncan, Marsh Seedless, and Silver Cluster varieties from Polk County, Fla., which were placed in storage February 12, 1920. Part of the fruit of each lot was placed directly in 32° F., and the rest of the three lots were placed in the curing room and maintained at a temperature of about 70° with a relative humidity around 60 per cent. Portions of the lots from the curing room were removed to 32° cold storage at intervals. The entire storage period was three months for all lots. The results of the experiment are shown in Table IV, in which are given the length of time in curing, the time in cold storage, and the percentage of pitting of the different lots. In these experiments the pitting is given as slight and bad pitting. Bad pitting is applied to pitting that would markedly injure the sale of the fruit. Slight pitting refers to pitting that while noticeable does not particularly injure the fruit for sale. It is at most a few spots usually small.

It is noticeable in Table IV that most of the control fruit that was placed directly in cold storage without curing is pitted and that there is a high percentage of bad pitting. In the Duncan, 6 per cent was good, while the poorest lot of cured fruit of this variety was about 90 per cent good. There was more pitting in the cured Silver Cluster than in the Duncan and somewhat more in the Marsh Seedless than in the Silver Cluster. The data obtained in this one storage experiment are hardly sufficient, however, to justify the conclusion that Duncan grapefruit store better than Silver Cluster and Marsh Seedless. The experiments, however, seem to show that the pitting can be controlled by proper curing before the fruit is placed at the low temperatures. The specific effect of this curing, by exposure to warm temperatures from one to six weeks, on the tissue of the peel so that the pitting is prevented has, of course, received little attention. Pitting has all the external appearance of injury considered to be due to *Colletotrichum gloeosporioides* (Penz.) by Rolfs, Fawcett, and Floyd (*13*) and figured by them. This fungus,

however, has a high optimum temperature, and it seemed highly improbable that its growth could be controlled by exposing to high temperatures and that it caused most damage at temperatures around 32° to 40° F. It was possible, however. This point was investigated by Dr. F. V. Rand, of the Laboratory of Plant Pathology, Bureau of Plant Industry. The results of this work are as yet unpublished. The following account, however, is based on Dr. Rand's work.

TABLE IV.—*Results of storage experiments with Duncan, Marsh Seedless, and Silver Cluster grapefruit*

DUNCAN

Number of fruits.	Number of days in curing room.	Number of days in cold storage.	Temperature of cold storage.	Number of good fruits.	Percentage of good fruit.	Number with slight pitting.	Percentage with slight pitting.	Number with bad pitting.	Percentage with bad pitting.
			°F.						
50	[a] 00	90	32	3	6	10	20	37	74
27	12	78	32	24	88.9	3	11.1	.....	.....
23	19	71	32	23	100	.....	.....	.....	.....
14	25	65	32	13	92.8	1	7.1	.....	.....
10	41	49	32	10	100	.....	.....	.....	.....

MARSH SEEDLESS

82	[a] 00	90	32	3	3.6	6	7.3	73	89
42	10	80	32	11	26.2	.....	.....	31	73.8
30	18	72	32	12	40	6	20	12	40
19	24	66	32	16	84.2	3	15.8	.....	.....
12	30	60	32	10	83.3	2	16.7	.....	.....

SILVER CLUSTER

46	[a] 00	90	32	23	50	.....	.....	23	50
16	9	81	32	14	87.5	2	12.5	.....	.....
17	18	72	32	15	88.2	2	11.8	.....	.....
20	25	65	32	17	85	3	15	.....	.....

[a] Controls placed directly in cold storage.

Cultures were made from the pits and from the tissue of the peel between the pits. Cultures were also made from the peel of cured fruits which had been in cold storage for three months after curing. The results are shown in Table V.

It is evident from Table V that Colletotrichum was almost universally present in the peel of these Florida grapefruits and that while it is usually to be found in the pit it is just as common in the normal peel of the pitted fruit or the cured fruit. It is, of course, impossible to assert from the evidence at hand that the fungus does not cause the breaking down of the peel. The cold storage might so affect the physiology of the peel as to make it susceptible to fungus attacks, while curing

and warm storage render it resistant. This, of course, is somewhat doubtful. The case is somewhat analagous to that cited by Winston (*15*) in regard to tear stain, which has up to now been considered to be due to *Colletotrichum gloeosporioides*, mainly because this fungus was usually found in cultures from the diseased areas. In the present work it seems fair to conclude that whether or not the fungus causes the pitting it is controlled at least to a large extent by curing before placing the fruit in cold storage.

TABLE V.—*Results of cultural experiments with pitted grapefruit and with fruit from same lot which was unpitted* [a]

Date.	Source.	Number of fruits.	Number of pieces of tissue.	Colletotrichum.	Cladosporium.	Alternaria.	Fusarium.	Penicillium.	Sterile.	Miscellaneous fungi.
July 7, 1920	Pits	6	96	33	31	1	.....	.....	31	.....
	Between pits	1	7	1	5	.....	.....	.....	1	.....
Aug. 9, 1920	Pits	2	33	17	.....	.....	.....	.....	16	.....
	Between pits	2	17	10	.....	.....	.....	.....	7	.....
Feb. 1, 1921	Pits	7	53	30	3	.....	.....	1	21	1
	Between pits	4	29	15	3	.....	.....	3	8	6
	Control	5	70	42	.....	.....	.....	4	22	4
Feb. 1, 1921 (not sterilized)	Pits	3	32	21	.....	.....	.....	21	.....	13
	Between pits	2	27	12	.....	.....	.....	11	.....	27
	Control	1	14	14	.....	.....	.....	10	.....	13
Feb. 3, 1921 (sterilized 3 minutes).	Pits	3	38	.....	.....	.....	.....	.....	38	.....
	Between pits	3	17	3	.....	.....	1	.....	13	.....
	Control	3	23	16	.....	.....	1	.....	6	.....
Feb. 3, 1921 (not sterilized)	Pits	4	38	38	.....	.....	2	.....	.....	33
	Between pits	2	24	15	.....	.....	.....	2	.....	7
	Control	2	20	17	.....	.....	.....	1	.....	3
Feb. 8, 1921	Pits	5	93	68	.....	6	7	.....	10	10
	Between pits	2	38	27	.....	3	.....	1	4	2
	Control	4	84	63	.....	5	5	.....	10	3

[a] Unless otherwise stated, all pieces of tissue were sterilized two minutes in 1 to 1,000 bichlorid solution and were washed three times in sterile tap water before pouring plates. The control fruits were without signs of the pitted spots under investigation. "Between pits" refers to sound tissue between the spots.

GENERAL DISCUSSION AND CONCLUSION

In the investigation of grapefruit storage described in the foregoing pages it has been brought out that in warm storage the percentage of acid calculated to the wet weight of the pulp increases markedly in two months' storage. There is evidence that this increase is not due entirely to loss of water from the pulp, but that there is an increase in the amount of acid present. There is evidence indicating that there may be a slight decrease in the sugar content in warm storage. In cold storage there is a decrease in the acidity very marked after four months in storage, while there is little change in the amount of total sugars present. A possible explanation of this difference in the behavior of the sugars and acids in warm and cold storage was pointed out. This phase of the problem deserves further attention. The investigations on the changes in the

fruit during development on the tree showed that the total sugar content increased while the acidity decreased, the increase in sugar content being very marked.

Fruit on the tree increases in palatability and food value. There is, of course, always danger that the seeds will sprout in the varieties containing seeds if the fruit remains on the tree too long. There is also danger that the fruit will drop or be shaken from the tree by high winds.

It is of interest to note that the behavior of the acids and sugars during growth and in cold storage is similar to the behavior of these constituents of some of the deciduous fruits—that is, it is apparently possible to remove the fruit from the tree after it is well along toward maturity and to ripen it in storage. The result will be an apparently sweeter fruit, due to loss of acidity and a reduced bitterness, the naringin or bitter principle breaking down in storage. A period in cold storage, then, renders the fruit more palatable. From the experiments detailed above it seems probable that the pitting of grapefruit can be controlled by curing at 70° F. before they are placed in cold storage. Investigations are in progress at the present time on this last-mentioned phase of the work.

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ABSORPTION OF COPPER FROM THE SOIL BY POTATO PLANTS

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RESULTS OF PREVIOUS INVESTIGATIONS

Some of the results obtained by a few investigators on the absorption of copper by plants and cells may be summarized as follows:

Schander [1] found that copper in a soluble form is a poison for plant cells of both high and low order.

Tschirch [2] believes that living plants are able to absorb copper through their roots and also through the epidermis of the leaves, the amount of copper absorbed being very small, however.

Haselhoff [3] stated that soluble copper salts are injurious to plants at a concentration of 10 mgm. of cupric oxid per liter. When soluble copper salts are added to the soil the plant materials, especially the potash and the lime, are dissolved and washed away, as a consequence of which the fertility of the soil is decreased. The action of copper sulphate is more severe on some crops than on others. The presence of calcium carbonate in the soil prevents or decreases the toxicity of solutions of copper sulphate.

True and Gies [4] have shown that when lime is used with copper sulphate solutions the toxicity of the copper is decreased. They state that when there is lime in the soil four times the amount of copper that can be allowed when no lime is found may be present in a soil without exerting a toxic action.

Forbes [5] found that corn grown in soil containing copper held most of the copper in the roots rather than in the tops. He states also that the toxicity of copper depends on the combination in which it exists in the soil, the physical characteristics of the soil, and the chemical composition of the soil, and on climatic and moisture conditions, as well as on the crop grown.

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[2] Tschirch, A. Das kupfer vom standpunkte der gerichtlichen chemie, toxicologie und hygiene. 138 p., 2 fig. Stuttgart. 1893. Bibliographical footnotes.

[3] Haselhoff, Emil. Ueber die schädigende wirkung von kupfersulfat und kupfernitrathaltigem wasser auf boden und pflanzen. *In* Landw. Jahrb., Bd. 21, p. 263–276, 2 pl. 1892.

[4] True, Rodney H., and Gies, William J. On the physiological action of some of the heavy metals in mixed solutions. *In* Bul. Torrey Bot. Club, v. 30, no. 7, p. 390–402. 1903.

[5] Forbes, R. H. Certain effects under irrigation of copper compounds upon crops. Ariz. Agr. Exp. Sta. Bul. 80, p. 145–238, 16 fig., 4 pl. (1 col.). Bibliography, p. 236–238.

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OBJECT OF PRESENT EXPERIMENTS

The experiments discussed in this paper were undertaken to determine what proportion of the copper present in standard Bordeaux spray, in Pickering's limewater Bordeaux spray, and in a solution of copper sulphate, of equal copper content, is absorbed by potato plants when the sprays or solution are applied directly to the soil in which the vines are growing. The comparative distribution of the absorbed copper in different parts of the potato plants was also studied.

The copper in the Pickering spray was in an insoluble form, basic copper sulphate, with no excess of lime present. The copper of the Bordeaux spray was in an insoluble form, with a large excess of lime present. The copper of the solution of copper sulphate was soluble. It was believed that a comparative study of these three sprays, containing copper in equal amounts, would show the extent to which the excess lime of Bordeaux spray is instrumental in preventing the absorption of copper by the roots of the potato plants, as well as the relation of the absorption of copper from a soluble copper compound to that from an insoluble copper compound when applied to the soil.

EXPERIMENTAL WORK

The tests were conducted on the Aroostook Farm of the Maine Agricultural Experiment Station, at Presque Isle, Me., on Caribou type soil. A single row, 8 feet long, of Norcross strain of the Green Mountain variety of Irish potato plants was used for each of four plots which were treated in the following manner: Plot 1, sprayed with standard Bordeaux, 3–3–50 formula, containing 0.75 per cent of copper sulphate; plot 2, sprayed with an "A" formula Pickering limewater Bordeaux spray, containing 0.70 per cent of copper sulphate; plot 3, sprayed with a solution containing 0.75 per cent of copper sulphate; and plot 4, a control plot, unsprayed.

The vines were 20 inches above ground when the first applications were made. At each application 1 gallon of the spray or solution was applied directly to the ground within 6 inches of the stems of eight potato plants in each plot, each vine thus receiving 1 pint of the solution to each treatment. An equal amount of water was applied to the roots of eight control plants at the time the other applications were made. Applications were made on July 27, August 8, August 17, August 24, and August 30, 1917.

PREPARATION OF SAMPLES

Vines and tubers from each of the four plots were taken for analysis at frequent intervals.

The vines from the various plots were dried in the air, then washed in running water and held for 30 seconds in a 4 per cent solution of hydrochloric acid, after which they were immediately washed in water and

finally in distilled water. The vines were next dried for 16 hours in an oven at 110° C. Separate analyses of leaves, stems, roots, and tubers were made.

Five or six tubers from each plot were thoroughly washed, rinsed in distilled water, and dried with a towel. The tubers were pared, passed through a grinder, well mixed, and transferred to a Mason jar with rubber and top. Care is necessary in securing a uniform sample of the ground tubers for analyses, as the water and solids separate very rapidly.

Samples of soil were taken 6 inches deep, near the roots of the treated plants, from the various plots at the time the plants were sampled. The soil samples were held in Mason jars with rubbers and tops until analyzed. Before analysis the stones and other foreign matter were removed from the samples.

DETERMINATION OF COPPER IN VINES AND TUBERS

From 5 to 10 gm. of the dried leaves and stems, and from 1 to 5 gm. of the roots were taken for copper analyses. The samples were ashed in 4-inch porcelain dishes, 30 cc. of 5 per cent nitri acid were added, and the whole was allowed to remain overnight. The solutions were filtered and washed, after which ammonia was added to faint alkalinity. They were brought to a boil, cooled, and made to volume, usually 150 cc. The precipitated iron and alumina were removed by filtration, and an aliquot of the filtrate was taken for the determination of copper.

TABLE I.—*Copper found in potato vines and tubers*[a]

Plants taken for analysis.	Parts analyzed.	Soil treated with Pickering spray (0.75 per cent $CuSO_4$).	Soil treated with Bordeaux spray (0.75 per cent $CuSO_4$).	Soil treated with $CuSO_4$ solution (0.75 per cent $CuSO_4$).	Control plot.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Aug. 8	Leaves	0.0045	.....	0.0065	0.0030
	Stem	.0015	0.0070	.0079	.0043
	Root	0	.0136	.0052	.0017
17	Leaves	.0097	.0100	.0069	.0089
	Stem	.0023	.0042	.0047	.0026
	Root	.0036	.0030	.0101	.0020
	Tubers	.0004	.....	.0001	.0001
24	Leaves	.0179	.0258	.0109	.0087
	Stem	.0029	.0048	.0067	.0022
	Root	.0128	.0104	.0130	.0010
	Tubers	.0001	.0001	.0001	.0002
Sept. 3	Leaves	.....	.....	.....	.....
	Stem	.0053	.0069	.0225	.0017
	Root	.0160	.0069	.0300	0
Average	Leaves	.0107	.0179	.0081	.0069
	Stem	.0030	.0057	.0104	.0027
	Root	.0081	.0085	.0146	.0012
	Tubers	.0002	.0001	.0001	.0002

[a] Analyses made on dry basis.

As a rule, 25-cc. aliquots were evaporated to dryness in 50-cc. porcelain dishes on the steam bath, and the residue was taken up in 5 cc. of distilled water. Two drops of acetic acid and 3 drops of 1 per cent solution of potassium ferrocyanid were added, and the color was immediately compared with that of standard solutions of copper sulphate which had been evaporated with ammonium nitrate and taken up in 5 cc. of distilled water.

Copper in the ground tubers was determined by the same procedure, using 50 gm. of the moist sample. The analytical data are recorded in Table I.

DETERMINATION OF COPPER IN SOILS

One hundred gm. of the well-mixed soil samples were treated with a mixture of 80 cc. of nitric acid and 20 cc. of sulphuric acid in large porcelain casseroles. The mixtures were heated on the steam bath and then on the hot plate until the nitric acid fumes were removed. The residues were extracted with 200 cc. of water, filtered, washed, and made to 500 cc. volume. After evaporation to 200 cc., the iron was precipitated with ammonia and the solutions were made to volume. They were next filtered and aliquots were made acid with hydrochloric acid, through which hydrogen sulphid was passed for 20 minutes, or until all the copper was precipitated. The precipitated copper after settling was filtered and dissolved in 10 cc. of nitric acid, the filter paper and precipitate being transferred together. Ammonia was added to faint alkalinity, and the solutions were evaporated to dryness in small porcelain dishes. The residues were taken up in 5 cc. of distilled water, two drops of acetic acid and three drops of 1 per cent potassium ferrocyanid were added, and the copper was estimated by colorimetric comparisons. In some cases after evaporation to dryness it was necessary to take up in water, filter, wash, and repeat the evaporation to remove precipitated material.

If present in large enough amounts copper may be determined electrolytically, by a method based on the procedure given by Forbes, Free, and Ross.[1]

The results of the analyses of the first and last samples of soil taken appear in Table II. This table gives also the results of a series of tests on the soil around the roots of potato plants which had been commercially sprayed with Bordeaux, with Pickering spray, and with a solution of copper sulphate, to determine whether any appreciable amounts of the copper occur in the soil beneath the sprayed vines.

[1] FORBES, R. H. CERTAIN EFFECTS UNDER IRRIGATION OF COPPER COMPOUNDS UPON CROPS. Ariz. Agr. Exp. Sta. Bul. 80, p. 145–238, 16 fig., 4 pl. (1 col.). 1916. Bibliography, p. 236–238. Part 3, Appendix: Methods of analysis, with the collaboration of E. E. Free and W. H. Ross, p. 229–235.

TABLE II.—*Copper found in soil*

SPRAYS APPLIED TO SOIL NEAR PLANT ROOTS

Sample No.	Date of sampling.	Description of samples.	Description of plots.	Total copper found in soil.
				P. p. m.
1	July 26	Samples taken before any copper was added to the soils.	Control	2
2			Pickering "A" formula	1
3			Bordeaux 3-3-50	2
4			$CuSO_4$ solution	2
5	Aug. 24	Samples taken just before last application of copper to soil.	Control	5
6			Pickering "A" formula	211
7			Bordeaux 3-3-50	256
8			$CuSO_4$ solution	250
9	Sept. 3	Samples taken after the last application of copper to the soil.	Control	2
10			Pickering "A" formula	225
11			Bordeaux 3-3-50	243
12			$CuSO_4$ solution	449

SPRAYS APPLIED TO VINES IN COMMERCIAL PRACTICE

A	July 16	Samples taken before any sprayings were made in 1917.	Control	1
B			Pickering "C" formula	1
C			Bordeaux 5-5-50	1
D			Pickering "A" formula	1
E	Aug. 31	Samples taken after last sprayings were made in 1917.	Control	2
F			Pickering "C" formula	2
G			Bordeaux 5-5-50	2
H			Pickering "A" formula	3

DISCUSSION OF RESULTS

VINES AND TUBERS

The leaves, stems, and roots of the plants from the soil receiving the Pickering spray showed an increased copper content with each successive analysis (Table I). The largest percentage of the copper was held by the leaves. The roots held an appreciable part of the copper, the amount increasing from 0 in the first sample to 0.0160 per cent in the sample taken on September 3. The tubers contained only minute amounts of copper.

The plants from the Bordeaux treated soil showed irregularities, particularly with respect to the copper content of the roots and stems. The leaves and stems contained more copper than those of the plants from the Pickering treated soil, while the roots contained less copper than the roots of the plants from the Pickering treated soil. The amounts of copper found in the tubers were small.

The vines grown in the soil treated with a solution of copper sulphate showed a marked progressive increase in copper content of the roots with each succeeding analysis. The leaves contained somewhat larger

amounts of copper than the stems, but not as much as the roots. The leaves contained less copper than the leaves of the plants grown on the Bordeaux or Pickering treated soils. The tubers from the plot treated with copper sulphate solution were as low in copper as those from the other plots.

The analyses of the various portions of the control plants showed the presence of copper, but in smaller amounts than in the plants grown on soil treated with the copper sprays.

The results of the copper absorption experiments indicate that the potato plants, with the exception of those grown in the soil receiving the solution of copper sulphate where the roots were distinctly injured, distributed the largest part of the absorbed copper to the leaves, while the roots and stems contained appreciable amounts of copper. In all normally sprayed potato plants the largest proportion of the copper is said to be found in the leaves.

The plants grown on the soil treated with a solution of copper sulphate were small and lacking in vigor. The roots had but few hairs, and showed other signs of injury. The large percentage of copper found in the roots, together with the small size of the roots, indicated some interference with the normal metabolism of the vines. The toxic effect of the soluble copper salt was exerted primarily on the roots of the plants. It was apparent that the soluble copper sulphate had injured the potato plants, while the insoluble copper compounds had not.

The vines from the Bordeaux plot contained a little more copper than the vines from the Pickering plot, indicating that the extra lime of the Bordeaux spray did not aid in preventing the absorption of copper by the plants.

SOIL

The results of the analyses of the first and last samples of soil taken show that no water-soluble copper was found in any of the samples examined. The amount of copper in the first set of samples (Table II, No. 1, 2, 3, and 4) which were taken before any copper had been added to the soils, is practically the same in all cases. The sets of samples taken before and after the final treatment of the plots show the presence of a large amount of copper in the samples receiving the copper treatments. This means that copper in an insoluble form may be present in the soil in marked amounts without exerting any apparent toxic action on the growth of potato plants.

But little copper was found in the soil as a result of spraying with copper sprays according to commercial practice.

On September 5, shortly after the last treatment of the soil, a few hills of potatoes were dug. The weights and number of the tubers, the percentage of decayed tubers, as well as the notes taken on the size and appearance of the vines are given in Table III.

TABLE III.—*Effect on potato tubers and vines of applications of sprays to soil*

Spray used.	Tubers.[a]			Stand and condition vines.
	Number and size.	Weight.		
		Total.	Average.	
		Ounces.	*Ounces.*	
Bordeaux (0.75 per cent $CuSO_4$).	3 (1 large and 2 small) in 1 hill.	8	2⅔	Normal.
Pickering (0.7 per cent $CuSO_4$).	8 (5 large and 3 small) in 2 hills.	29	3⅝	Do.
Copper-sulphate solution (0.75 per cent $CuSO_4$).	17 (all small) in 4 hills.	23	1½	Small and stunted.
Unsprayed (control).	8 (3 large, 3 medium, and 2 rotten) in 2 hills.	24	3	Normal stand; blight.

[a] Rot found only on control tubers.

These data show that the solution of copper sulphate had a very disastrous effect on the growth and yield of the tubers. The only decayed tubers found were obtained from the unsprayed plot. These results are so few that they can be considered only as suggestive.

SUMMARY

Potato plants grown in soil treated with insoluble copper compounds contained more copper in the leaves than in the stems, while but little copper was found in the roots. The tubers showed only traces of copper.

When the soil was treated with the copper sulphate solution, the roots were injured and the normal metabolism of the vines was disturbed. The tubers from these vines were small and the vines stunted. The roots of these plants held more copper than the leaves.

The soluble copper sulphate added directly to the soil caused injury to the plants, while the insoluble copper compounds of the sprays did not. The excess lime of the Bordeaux spray did not reduce the amount of copper absorbed by the plants compared with the plants grown on the Pickering plot.

Practically the same amounts of copper were found in all the soil samples tested. Samples of soil from sprayed potato fields showed but minute amounts of copper.

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